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$  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:22:40 ON 26 JUN 2003

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 16:22:50 ON 26 JUN 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s thymosin(2a)beta
FILE 'MEDLINE'
          1718 THYMOSIN
        453335 BETA
L1         350 THYMOSIN(2A)BETA

FILE 'SCISEARCH'
          1474 THYMOSIN
        597170 BETA
L2         425 THYMOSIN(2A)BETA

FILE 'LIFESCI'
           420 THYMOSIN
        146426 BETA
L3         119 THYMOSIN(2A)BETA

FILE 'BIOTECHDS'
            75 THYMOSIN
         34187 BETA
L4          18 THYMOSIN(2A)BETA

FILE 'BIOSIS'
          2063 THYMOSIN
        599990 BETA
L5         495 THYMOSIN(2A)BETA

FILE 'EMBASE'
          1885 THYMOSIN
        501332 BETA
L6         297 THYMOSIN(2A)BETA

FILE 'HCAPLUS'
          1820 THYMOSIN
       1195922 BETA
L7         578 THYMOSIN(2A)BETA

FILE 'NTIS'
            15 THYMOSIN
         19963 BETA
L8            1 THYMOSIN(2A)BETA

FILE 'ESBIOBASE'
           237 THYMOSIN
        168783 BETA
L9         133 THYMOSIN(2A)BETA

FILE 'BIOTECHNO'
           506 THYMOSIN
        173923 BETA
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L10         174 THYMOSIN(2A)BETA

FILE 'WPIDS'
            197 THYMOSIN
         101256 BETA
L11          35 THYMOSIN(2A)BETA

TOTAL FOR ALL FILES
L12        2625 THYMOSIN(2A) BETA

=> s l12(5a)gene/q
FILE 'MEDLINE'
L13          48 L1 (5A)GENE/Q

FILE 'SCISEARCH'
L14          47 L2 (5A)GENE/Q

FILE 'LIFESCI'
L15          29 L3 (5A)GENE/Q

FILE 'BIOTECHDS'
L16           9 L4 (5A)GENE/Q

FILE 'BIOSIS'
L17          87 L5 (5A)GENE/Q

FILE 'EMBASE'
L18          47 L6 (5A)GENE/Q

FILE 'HCAPLUS'
L19         197 L7 (5A)GENE/Q

FILE 'NTIS'
L20           0 L8 (5A)GENE/Q

FILE 'ESBIOBASE'
L21          16 L9 (5A)GENE/Q

FILE 'BIOTECHNO'
L22          42 L10(5A)GENE/Q

FILE 'WPIDS'
L23           8 L11(5A)GENE/Q

TOTAL FOR ALL FILES
L24         530 L12(5A) GENE/Q

=> s l24 not 2001-2003/py
FILE 'MEDLINE'
        1274341 2001-2003/PY
L25          42 L13 NOT 2001-2003/PY

FILE 'SCISEARCH'
        2320604 2001-2003/PY
L26          40 L14 NOT 2001-2003/PY

FILE 'LIFESCI'
         223576 2001-2003/PY
L27          25 L15 NOT 2001-2003/PY

FILE 'BIOTECHDS'
          46337 2001-2003/PY
L28           7 L16 NOT 2001-2003/PY
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FILE 'BIOSIS'
       1237159 2001-2003/PY
L29          70 L17 NOT 2001-2003/PY

FILE 'EMBASE'
       1052361 2001-2003/PY
L30          39 L18 NOT 2001-2003/PY

FILE 'HCAPLUS'
       2443656 2001-2003/PY
L31         107 L19 NOT 2001-2003/PY

FILE 'NTIS'
         34498 2001-2003/PY
L32           0 L20 NOT 2001-2003/PY

FILE 'ESBIOBASE'
        678852 2001-2003/PY
L33          10 L21 NOT 2001-2003/PY

FILE 'BIOTECHNO'
        273208 2001-2003/PY
L34          33 L22 NOT 2001-2003/PY

FILE 'WPIDS'
       2283321 2001-2003/PY
L35           1 L23 NOT 2001-2003/PY

TOTAL FOR ALL FILES
L36         374 L24 NOT 2001-2003/PY

=> dup rem l36
PROCESSING COMPLETED FOR L36
L37          153 DUP REM L36 (221 DUPLICATES REMOVED)

=> d tot

L37  ANSWER 1 OF 153  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI   Human thymosin beta15 gene, protein and uses thereof.
SO   Official Gazette of the United States Patent and Trademark Office Patents,
     (Jan. 25, 2000) Vol. 1230, No. 4, pp. No pagination. e-file.
     ISSN: 0098-1133.
AU   Zetter, Bruce R. (1); Bao, Lere
AN   2000:324226  BIOSIS

L37  ANSWER 2 OF 153  HCAPLUS  COPYRIGHT 2003 ACS
TI   Identification of iron responsive genes by screening cDNA libraries from
     suppression subtractive hybridization with antisense probes from three
     iron conditions
SO   Nucleic Acids Research (2000), 28(8), 1802-1807
     CODEN: NARHAD; ISSN: 0305-1048
AU   Ye, Zheng; Connor, James R.
AN   2000:336315  HCAPLUS
DN   133:262126

L37  ANSWER 3 OF 153  HCAPLUS  COPYRIGHT 2003 ACS
TI   Gene expression profiles in thyroid carcinomas
SO   British Journal of Cancer (2000), 83(11), 1495-1502
     CODEN: BJCAAI; ISSN: 0007-0920
AU   Takano, T.; Hasegawa, Y.; Matsuzuka, F.; Miyauchi, A.; Yoshida, H.;
     Higashiyama, T.; Kuma, K.; Amino, N.
AN   2000:899969  HCAPLUS
DN   134:351381
```

L37 ANSWER 4 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Positive and negative haematopoietic cytokines produced by bone marrow endothelial cells
SO Cytokine (2000), 12(7), 1017-1023
CODEN: CYTIE9; ISSN: 1043-4666
AU Li, Wei Min; Huang, Wei Qi; Huang, Yan Hong; Jiang, De Zhao; Wang, Qi Ru
AN 2000:449519 HCAPLUS
DN 133:218066

L37 ANSWER 5 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 1
TI Identification of known and novel genes in activated monocytes from patients with rheumatoid arthritis
SO ARTHRITIS AND RHEUMATISM, (APR 2000) Vol. 43, No. 4, pp. 775-790.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621.
ISSN: 0004-3591.
AU Stuhlmuller B (Reprint); Ungethum U; Scholze S; Martinez L; Backhaus M; Kraetsch H G; Kinne R W; Burmester G R
AN 2000:291680 SCISEARCH

L37 ANSWER 6 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Differential gene expression in cryptogenic acute liver failure.
SO Hepatology, (October, 2000) Vol. 32, No. 4 Pt. 2, pp. 610A. print.
Meeting Info.: 51st Annual Meeting and Postgraduate Courses of the American Association for the Study of Liver Diseases Dallas, Texas, USA October 27-31, 2000 American Association for the Study of Liver Diseases . ISSN: 0270-9139.
AU Heringlake, Stefan (1); Sauer, Arik (1); Tillmann, Hans L.; Manns, Michael P.
AN 2000:495341 BIOSIS

L37 ANSWER 7 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Genomic analysis of metastasis reveals an essential role for RhoC
SO Nature (London) (2000), 406(6795), 532-535
CODEN: NATUAS; ISSN: 0028-0836
AU Clark, Edwin A.; Golub, Todd R.; Lander, Eric S.; Hynes, Richard O.
AN 2000:613434 HCAPLUS
DN 133:250523

L37 ANSWER 8 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI The comparison of .beta.-thymosin homologues among metazoa supports an arthropod-nematode clade
SO Journal of Molecular Evolution (2000), 51(4), 378-381
CODEN: JMEVAU; ISSN: 0022-2844
AU Manuel, Michael; Kruse, Michael; Muller, Werner E. G.; Le Parco, Yannick
AN 2000:831436 HCAPLUS
DN 134:83743

L37 ANSWER 9 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Genes upregulated in the human trabecular meshwork in response to elevated intraocular pressure.
SO IOVS, (Feb., 2000) Vol. 41, No. 2, pp. 352-361.
AU Gonzalez, Pedro; Epstein, David L.; Borras, Teresa (1)
AN 2000:113849 BIOSIS

L37 ANSWER 10 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 2
TI Review of thymic hormones in cancer diagnosis and treatment
SO INTERNATIONAL JOURNAL OF IMMUNOPHARMACOLOGY, (APR 2000) Vol. 22, No. 4, pp. 261-273.
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
ISSN: 0192-0561.
AU Bodey B (Reprint); Bodey B; Siegel S E; Kaiser H E
AN 2000:264197 SCISEARCH

L37 ANSWER 11 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Functional analysis of human thymosin beta4 promoter in aortic valve interstitial cells.
SO American Journal of Human Genetics, (October, 2000) Vol. 67, No. 4 Supplement 2, pp. 180. print.
Meeting Info.: 50th Annual Meeting of the American Society of Human Genetics Philadelphia, Pennsylvania, USA October 03-07, 2000 American Society of Human Genetics
. ISSN: 0002-9297.
AU Li, Q. Y. (1); Lafferty, R. P. (1); Levy, R. J. (1)
AN 2000:503857 BIOSIS

L37 ANSWER 12 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Differential gene expression between ALK-positive and ALK-negative anaplastic large cell lymphoma detected by suppression subtractive hybridization.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 168b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
AU Villalva, Claire (1); Zenou, Reine Claude (1); Delsol, Georges (1); Brousset, Pierre (1)
AN 2001:299305 BIOSIS

L37 ANSWER 13 OF 153 MEDLINE DUPLICATE 3
TI Characterization of a 5'-flanking region supporting the transcription of mouse thymosin beta-4 in mouse NIH3T3 cells.
SO MOLECULAR AND CELLULAR BIOCHEMISTRY, (2000 Jan) 203 (1-2) 163-7.
Journal code: 0364456. ISSN: 0300-8177.
AU Su Y; Chang S L; Hsiao H L
AN 2000186940 MEDLINE

L37 ANSWER 14 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI The pattern of gene expression in human myeloid progenitor cells.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 121b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
AU Lee, Sanggyu (1); Clark, Terry; Chen, Jianjun (1); Zhou, Guolin (1); Rowley, Janet D. (1); Wang, San Ming (1)
AN 2001:290155 BIOSIS

L37 ANSWER 15 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Aged human muscle demonstrates an altered gene expression profile consistent with an impaired response to exercise
SO Mechanisms of Ageing and Development (2000), 120(1-3), 45-56
CODEN: MAGDA3; ISSN: 0047-6374
AU Jozsi, A. C.; Dupont-Versteegden, E. E.; Taylor-Jones, J. M.; Evans, W. J.; Trappe, T. A.; Campbell, W. W.; Peterson, C. A.
AN 2000:829837 HCAPLUS
DN 134:160670

L37 ANSWER 16 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 4
TI Molecular cloning and structural characterization of the rat **thymosin beta** 15 **gene**
SO GENE, (30 DEC 2000) Vol. 260, No. 1-2, pp. 37-44.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0378-1119.
AU Bao L; Zetter B R (Reprint)
AN 2001:36836 SCISEARCH

L37 ANSWER 17 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Gene expression profiles of the collecting duct in the mouse renal inner medulla
SO Kidney International (2000), 57(1), 19-24
CODEN: KDYIA5; ISSN: 0085-2538
AU Takenaka, Masaru; Imai, Enyu; Nagasawa, Yasuyuki; Matsuoka, Yasuko; Moriyama, Toshiki; Kaneko, Tetsuya; Hori, Masatsugu; Kawamoto, Shoko; Okubo, Kousaku
AN 2000:297676 HCAPLUS
DN 133:133044

L37 ANSWER 18 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Identification of differentially expressed genes in cardiac hypertrophy by analysis of expressed sequence tags
SO Genomics (2000), 66(1), 1-14
CODEN: GNMCEP; ISSN: 0888-7543
AU Hwang, David M.; Dempsey, Adam A.; Lee, Cheuk-Yu; Liew, Choong-Chin
AN 2000:376177 HCAPLUS
DN 133:248010

L37 ANSWER 19 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Analysis of prolactin-modulated gene expression profiles during the Nb2 cell cycle using differential screening techniques
SO GenomeBiology [online computer file] (2000), 1(4), No pp. given
CODEN: GNBLFW; ISSN: 1465-6914
URL: http://www.genomebiology.com/retriever.asp?url=/2000/1/4/research/0008
AU Bole-Feysot, Christine; Perret, Eric; Roustan, Paul; Bouchard, Brigitte; Kelly, Paul A.
AN 2001:217568 HCAPLUS
DN 136:1220

L37 ANSWER 20 OF 153 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New thymosin promoter useful in the treatment of breast, pancreas and prostate cancer;
vector-mediated thymosin-beta-15 promoter expression in host cell, DNA primer and DNA probe, used for e.g. mamma cancer gene therapy
AU Zetter B R; Bao L
AN 1999-08735 BIOTECHDS
PI WO 9919485 22 Apr 1999

L37 ANSWER 21 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Determination of p53-controlled genes (via SAGE) and their uses in cancer diagnosis
SO PCT Int. Appl., 32 pp.
CODEN: PIXXD2
IN Madden, Stephen L.; Galella, Elizabeth A.; Bertelsen, Arthur H.; Beaudry, Gary A.
AN 1999:48825 HCAPLUS
DN 130:134943

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9901581	A1	19990114	WO 1998-US13903	19980702
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9883829	A1	19990125	AU 1998-83829	19980702

L37 ANSWER 22 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Suppressive subtractive hybridization reveals differential expression of serglycin, sorcin, bone marrow proteoglycan and prostate-tumor-inducing gene I (PTI-1) in drug-resistant and sensitive tumor cell lines of haematopoietic origin

SO European Journal of Cancer (1999), 35(12), 1735-1742
CODEN: EJCAEL; ISSN: 0959-8049
AU Beyer-Sehlmeyer, G.; Hiddemann, W.; Wormann, B.; Bertram, J.
AN 1999:778974 HCAPLUS
DN 132:231586

L37 ANSWER 23 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Identification of AUF-1 ligands reveals vast diversity of early response gene mRNAs
SO Nucleic Acids Research (1999), 27(6), 1464-1472
CODEN: NARHAD; ISSN: 0305-1048
AU Bhattacharya, Saswati; Giordano, Tony; Brewer, Gary; Malter, James S.
AN 1999:213440 HCAPLUS
DN 131:69812

L37 ANSWER 24 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Expression of thymosin .beta.4 messenger RNA in normal and kainate-treated rat forebrain
SO Neuroscience (Oxford) (1999), 90(4), 1433-1444
CODEN: NRSCDN; ISSN: 0306-4522
AU Carpintero, P.; Anadon, R.; Diaz-Regueira, S.; Gomez-Marquez, J.
AN 1999:256181 HCAPLUS
DN 131:30098

L37 ANSWER 25 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 5
TI beta-Thymosin is required for axonal tract formation in developing zebrafish brain.
SO Development (Cambridge), (April, 1999) Vol. 126, No. 7, pp. 1365-1374.
ISSN: 0950-1991.
AU Roth, Lukas W. A.; Bormann, Peter; Bonnet, Annick; Reinhard, Eva (1)
AN 1999:248033 BIOSIS

L37 ANSWER 26 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Serial analysis of gene expression in human monocytes and macrophages
SO Blood (1999), 94(3), 837-844
CODEN: BLOOAW; ISSN: 0006-4971
AU Hashimoto, Shin-Ichi; Suzuki, Takuji; Dong, Hong-Yan; Yamazaki, Nobuyuki; Matsushima, Kouji
AN 1999:494079 HCAPLUS
DN 131:270730

L37 ANSWER 27 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Identifying differentially expressed genes following transient cerebral ischemia in the rat.
SO Society for Neuroscience Abstracts., (1999) Vol. 25, No. 1-2, pp. 825.
Meeting Info.: 29th Annual Meeting of the Society for Neuroscience. Miami Beach, Florida, USA October 23-28, 1999 Society for Neuroscience
. ISSN: 0190-5295.
AU Meloni, B. P. (1); Majda, B. T. (1); Rixon, N. K. (1); Knuckey, N. W. (1)
AN 2000:136467 BIOSIS

L37 ANSWER 28 OF 153 MEDLINE DUPLICATE 6
TI **Thymosin beta**-10 **gene** overexpression is a general event in human carcinogenesis.
SO AMERICAN JOURNAL OF PATHOLOGY, (1999 Sep) 155 (3) 799-804.
Journal code: 0370502. ISSN: 0002-9440.
AU Santelli G; Califano D; Chiappetta G; Vento M T; Bartoli P C; Zullo F; Trapasso F; Viglietto G; Fusco A
AN 1999419276 MEDLINE

L37 ANSWER 29 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Gene expression profiles of human fetal nasopharyngeal tissue
SO Shengwu Huaxue Yu Shengwu Wuli Xuebao (1999), 31(6), 711-714

CODEN: SHWPAU; ISSN: 0582-9879
AU He, Zhi-Wei; Xu, Liang-Guo; Xie, Lu; Zhang, Ling; Lan, Ke; Ren, Cai-Ping; Yao, Kai-Tai
AN 2000:6679 HCAPLUS
DN 132:178466

L37 ANSWER 30 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Genes expressed during the differentiation of pancreatic AR42J cells into insulin-secreting cells
SO Diabetes (1999), 48(2), 304-309
CODEN: DIAEAZ; ISSN: 0012-1797
AU Mashima, Hirosato; Yamada, Shirou; Tajima, Tomoko; Seno, Masaharu; Yamada, Hidenori; Takeda, Jun; Kojima, Itaru
AN 1999:95648 HCAPLUS
DN 130:235348

L37 ANSWER 31 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI The study on thymosin beta-10 highly expressed in renal cell carcinoma
SO Aichi Ika Daigaku Igakkai Zasshi (1999), 27(2), 159-166
CODEN: AIDZAC; ISSN: 0301-0902
AU Akahori, Masachika
AN 1999:759786 HCAPLUS
DN 132:21684

L37 ANSWER 32 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 7
TI Expression of the **thymosin beta**(10) **gene** in normal and kainic acid-treated rat forebrain
SO MOLECULAR BRAIN RESEARCH, (18 JUN 1999) Vol. 70, No. 1, pp. 141-146. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0169-328X.
AU Carpintero P; Anadon R; GomezMarquez J (Reprint)
AN 1999:502596 SCISEARCH

L37 ANSWER 33 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Genes differentially expressed in medulloblastoma and fetal brain
SO Physiological Genomics [online computer file] (1999), 1, 83-91
CODEN: PHGEFP; ISSN: 1094-8341
URL: http://physiolgenomics.physiology.org/cgi/reprint/1/2/83.pdf
AU Michiels, E. M. C.; Oussoren, E.; Van Groenigen, M.; Pauws, E.; Bossuyt, P. M. M.; Voute, P. A.; Baas, F.
AN 2000:527842 HCAPLUS
DN 134:261755

L37 ANSWER 34 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Thymosin beta-10 (Tbeta-10) and ICAM-1 genes are differentially expressed in early passage versus senescent human microvascular dermal endothelial cells (HMVEC) and are regulated by VPF/VEGF.
SO Molecular Biology of the Cell, (Nov., 1999) Vol. 10, No. SUPPL., pp. 60a. Meeting Info.: 39th Annual Meeting of the American Society for Cell Biology Washington, D.C., USA December 11-15, 1999 The American Society for Cell Biology
. ISSN: 1059-1524.
AU Vasile, Eliza (1); Tomita, Yasuhiko (1); Brown, Larry (1); Kocher, Olivier (1); Dvorak, Harold F. (1)
AN 2000:27699 BIOSIS

L37 ANSWER 35 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Expressed-sequence-tag approach to identify differentially expressed genes following peripheral nerve axotomy
SO Molecular Brain Research (1999), 64(1), 34-40
CODEN: MBREE4; ISSN: 0169-328X
AU Tanabe, Katsuhisa; Nakagomi, Saya; Kiryu-Seo, Sumiko; Namikawa, Kazuhiko; Imai, Yuji; Ochi, Takahiro; Tohyama, Masaya; Kiyama, Hiroshi

AN 1999:17247 HCAPLUS
DN 130:195259

L37 ANSWER 36 OF 153 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Human thymosin beta-15;
recombinant protein, ribozyme and antisense oligonucleotide for use in cancer therapy
AU Zetter B R; Bao L
AN 1998-05510 BIOTECHDS
PI US 5721337 24 Feb 1998

L37 ANSWER 37 OF 153 MEDLINE DUPLICATE 8
TI **Thymosin beta**-10 **gene** overexpression correlated with the highly malignant neoplastic phenotype of transformed thyroid cells in vivo and in vitro.
SO CANCER RESEARCH, (1998 Feb 15) 58 (4) 823-8.
Journal code: 2984705R. ISSN: 0008-5472.
AU Califano D; Monaco C; Santelli G; Giuliano A; Veronese M L; Berlingieri M T; de Franciscis V; Berger N; Trapasso F; Santoro M; Viglietto G; Fusco A
AN 1998143572 MEDLINE

L37 ANSWER 38 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Thymosin .beta.15 expression in tumor cell lines with varying metastatic potential
SO Clinical & Experimental Metastasis (1998), 16(3), 227-233
CODEN: CEXMD2; ISSN: 0262-0898
AU Bao, Lere; Loda, Massimo; Zetter, Bruce R.
AN 1998:354317 HCAPLUS
DN 129:107387

L37 ANSWER 39 OF 153 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI DNA encoding thymosin-beta-15;
recombinant protein and antisense sequence for use in cancer therapy and DNA probe and antibody for use in cancer diagnosis
AU Zetter B R; Bao L
AN 1997-12145 BIOTECHDS
PI US 5663071 2 Sep 1997

L37 ANSWER 40 OF 153 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI DNAs encoding human NB-thymosin-beta and their fragments;
DNA probe and polymerase chain reaction.DNA primer for glioma diagnosis
AN 1997-11743 BIOTECHDS
PI JP 09191881 29 Jul 1997

L37 ANSWER 41 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Transcriptional regulation of the **gene** coding for mouse **thymosin beta**-4
SO FASEB JOURNAL, (31 JUL 1997) Vol. 11, No. 9, Supp. [S], pp. 1250-1250.
Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
ISSN: 0892-6638.
AU Su Y (Reprint); Hsiao H L; Chang S L
AN 1998:406717 SCISEARCH

L37 ANSWER 42 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Transcriptional regulation of the **gene** coding for mouse **thymosin beta**-4.
SO FASEB Journal, (1997) Vol. 11, No. 9, pp. A1070.
Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997
ISSN: 0892-6638.

AU Su, Y.; Hsiao, H. L.; Chang, S. L.
AN 1997:420514 BIOSIS

L37 ANSWER 43 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Changing patterns of gene expression in developing mouse kidney, as probed by differential mRNA display combined with cDNA library screening
SO Kidney International (1997), 51(3), 920-925
CODEN: KDYIA5; ISSN: 0085-2538
AU Rosenblum, Norman D.; Yager, Thomas D.
AN 1997:212623 HCAPLUS
DN 126:207914

L37 ANSWER 44 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Analysis and comparison of partial sequences of clones from a taste-bud enriched cDNA library
SO Journal of Dental Research (1997), 76(4), 831-838
CODEN: JDREAF; ISSN: 0022-0345
AU Hoon, M. A.; Ryba, N. J. P.
AN 1997:304880 HCAPLUS
DN 127:1555

L37 ANSWER 45 OF 153 MEDLINE DUPLICATE 11
TI A novel beta-thymosin from the sea urchin: extending the phylogenetic distribution of beta-thymosins from mammals to echinoderms.
SO JOURNAL OF PEPTIDE SCIENCE, (1997 Jul-Aug) 3 (4) 282-90.
Journal code: 9506309. ISSN: 1075-2617.
AU Stoeva S; Horger S; Voelter W
AN 97408127 MEDLINE

L37 ANSWER 46 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI .beta.-Thymosins from marine invertebrates: primary structure and interaction with actin
SO Cell Motility and the Cytoskeleton (1997), 38(2), 163-171
CODEN: CMCYEO; ISSN: 0886-1544
AU Safer, Daniel; Chowrashi, Prokash K.
AN 1997:678345 HCAPLUS
DN 127:329057

L37 ANSWER 47 OF 153 HCAPLUS COPYRIGHT 2003 ACS
TI Chemical and biological evaluation of .beta.-thymosins
SO Aktuelle Onkologie (1997), Volume Date 1996, 96(Thymic Peptides in Preclinical and Clinical Medicine), 1-12
CODEN: AKONFG; ISSN: 0174-2744
AU Voelter, W.; Stoeva, S.; Kapurniotu, A.; Tsitsiloni, O. E.; Echner, H.; Grubler, G.; Schick, M.; Lehmann, R.
AN 1997:809425 HCAPLUS
DN 128:84450

L37 ANSWER 48 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI **Thymosin beta** 4 **gene** knockout in embryonic stem cells.
SO MOLECULAR BIOLOGY OF THE CELL, (DEC 1996) Vol. 7, Supp. [S], pp. 2193-2193.
Publisher: AMER SOC CELL BIOL, PUBL OFFICE 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
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L37 ANSWER 121 OF 153 HCAPLUS COPYRIGHT 2003 ACS
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L37 ANSWER 129 OF 153 HCAPLUS COPYRIGHT 2003 ACS
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L37 ANSWER 151 OF 153 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: USXXAM
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AN 1982:98506 HCAPLUS
DN 96:98506

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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L37 ANSWER 152 OF 153 MEDLINE DUPLICATE 58
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L37 ANSWER 153 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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AN 1982:240033 BIOSIS

=> save temp l37 thymosin/a
ANSWER SET L37 HAS BEEN SAVED AS 'THYMOSIN/A'

=> d ab 45-47,51,56,58,59,69,73,90,99,118,129,136,141

L37 ANSWER 45 OF 153 MEDLINE DUPLICATE 11
AB The study of the phylogenetic distribution of the beta-thymosin family is important to elucidate its biological function further. A new thymosin, designated as thymosin beta 14, consisting of 40 amino acid residues and with a molecular weight of 4537 Da as determined by ion spray mass spectrometry, was isolated from the sea urchin. The N-terminus of this polypeptide is blocked by an acetyl group as found by matrix-assisted laser desorption mass spectrometric and amino acid analysis. The primary structure was elucidate by Edman degradation of the HPLC-purified thymosin beta 14 fragments produced by digestion with endoproteinase Asp-N and trypsin. **Sequence** comparison reveals that **thymosin beta** 14 is 73% homologous to thymosin beta 4, obtained from calf thymus. By isolating and characterising the structure of thymosin beta 14 from the sea urchin, an invertebrate, substantial knowledge about the phylogenetic distribution and evolution of beta-thymosins is gained.

L37 ANSWER 46 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB The .beta.-thymosins are distributed throughout the vertebrate phyla, and all known vertebrate .beta.-thymosins bind actin monomers. To det. whether .beta.-thymosin-like peptides function as actin-binding proteins in invertebrates, we fractionated perchloric acid exts. of the gonads of both the sea urchin, Arbacia punctulata, and the scallop, Argopecten

irradians, and screened the fractions for proteins which could be crosslinked to actin. In each case a peptide was isolated which crosslinks to actin from both rabbit skeletal muscle and scallop cross-striated adductor muscle; both peptides were sequenced and each was found to consist of 40 amino acid residues, compared with 41-43 residues for the vertebrate .beta.-thymosins. The sequences of the scallop and sea urchin .beta.-thymosins are 80% identical to each other, 75% identical to residues 1-40 of thymosin .beta.4, and 72-80% identical to residues 1-40 of other vertebrate .beta.-thymosins. The sea urchin peptide was found to inhibit actin polymn. and nucleotide exchange. The affinity of the sea urchin peptide for rabbit muscle actin is apparently lower than that of thymosin .beta.4, since about twice the concn. of sea urchin peptide is required to give inhibition of actin polymn. or nucleotide exchange equiv. to thymosin .beta.4.

L37 ANSWER 47 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB A review, with 30 refs. describing the isolation, sequence detn. and phylogenetic tree of .beta.-thymosins. The authors also discuss the synthesis of .beta.-thymosins, immunohistochem. studies, and different biol. effects of .beta.-thymosins.

L37 ANSWER 51 OF 153 MEDLINE DUPLICATE 12
AB Thymosin beta 4 (T beta 4) is an actin monomer sequestering protein that may have a critical role in modulating the dynamics of actin polymerization and depolymerization in nonmuscle cells. Its regulatory role is consistent with the many examples of transcriptional regulation of T beta 4 and of tissue-specific expression. Furthermore, lymphocytes have a unique T beta 4 transcript relative to the ubiquitous transcript found in many other tissues and cells. To determine how T beta 4 gene expression is regulated and how the alternative transcripts are derived, we cloned the mouse T beta 4 gene. We established that there is a single mouse T beta 4 gene and found that the lymphoid-specific transcript is generated by extending the ubiquitous exon 1 with an alternate downstream splice site. The transcription start site is defined by primer extension analysis, and the 5'-flanking region has many of the characteristics of a promoter. It is pyrimidine-rich and contains typical promoter elements, including a GC box, an initiator site, and consensus transcription factor binding sites. The mouse T beta 4 gene locus (Ptmb4) is located by interspecific backcross mapping to the distal region of the mouse X chromosome, linked to Btk and Gja6.

L37 ANSWER 56 OF 153 MEDLINE DUPLICATE 13
AB The relationship between the conformation of a peptide in solution and its interaction capacity is generally unclear. Trifluoroethanol (TFE), which stabilizes alpha-helical conformations, can be used to induce definite folding in synthetic peptides. The N-terminal part of **thymosin beta** 4, including the 5-20 **sequences**, is implicated in binding to monomeric actin. The corresponding peptide was synthesized and its conformation studied by CD. The peptide is unstructured in solution, and becomes folded at medium TFE concentrations, below 30%. In contrast, TFE does not significantly modify the conformation of monomeric actin which conserves its intrinsic properties, such as gelsolin interaction and DNase-I inactivation. We report here that the apparent affinity of the synthetic peptide to monomeric actin is increased by an order of magnitude in the presence of TFE, which implies that the peptide adopts a folded conformation needed for accurate interaction.

L37 ANSWER 58 OF 153 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 14
AB Gelsolin and thymosin beta 4 appear to be two important actin-associated proteins involved in the regulation of actin polymerization. It has been widely demonstrated that thymosin is the major cellular actin-sequestering factor shifting the polymerization equilibrium of actin towards a monomeric state. At the same time gelsolin, a Ca2+ and inositol phosphate sensitive protein, regulates actin filament length. The

interactions of these two proteins with actin are rather complex and require the participation of several complementary peptide sequences. We have identified a common motif, (I, V)EKFD, in the two proteins in the functional **sequences** so far examined. Gelsolin- and **thymosin beta** 4-related peptides including the common motif were synthesized and their structural and functional properties studied. These two sequences exert a major inhibitory effect on salt-induced actin polymerization. We used circular dichroism and Fourier-transform infrared spectroscopy to show that the two synthetic peptides present some secondary structure in solution. As far as the peptide derived from the thymosin sequence was concerned, alpha-helical structure was induced by trifluoroethanol as observed with the full-length molecule. These experiments underscore the importance of the conformational state of peptide fragments in their biological activities. ELISA and fluorescence measurements have been used to identify the binding regions of these fragments to a C-terminal region (subdomain 1) of the actin sequence. Our results also emphasize the relationship between the propensity of small sequences to form secondary structures and their propensity for biological activity as related to actin interaction and inhibition of actin polymerization. (C) Munksgaard 1996.

L37 ANSWER 59 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB We have tried to further the understanding of the process of endothelial cell differentiation by detg. the identity of some of the early genes by subtractive cDNA cloning and sequencing. We were very successful and have identified several genes. Some of the genes that were found to be upregulated when examd. in Northern blots were calmodulin, tubulin, a collagen-like gene, ribosomal proteins, human elongation and splicing factors, several novel **genes**, and **thymosin** **beta**.4. Thymosin .beta.4, was increased 5-fold. Nothing is known about the expression or the regulation of thymosin .beta.4 in endothelial cells. Recently we reported strong evidence that this polypeptide is involved in the regulation of endothelial cell differential because: immunostaining localized thymosin .beta.4 in vivo to growing and mature vessels, endothelial cells transfected with thymosin .beta.4 showed increased attachment and spreading on matrix components, and an accelerated rate of tube formation on Matrigel, and direct addn. of purified thymosin .beta.4 protein to endothelial cells in culture induced greater tube formation and promoted actin cytoskeletal changes. The prepn. of antisense oligos to thymosin .beta.4 blocked thymosin protein synthesis and tube formation in vitro. The addn. of purified thymosin .beta.4 protein to endothelial cells in culture increased their attachment to matrix proteins, enhanced tube formation but inhibited cell proliferation. The results suggest that thymosin .beta.4 is induced and possibly involved in differentiating endothelial cells and is likely to play a role in vessel formation. Our study is the first study that illustrates the expression of thymosin .beta.4 is involved in endothelial cell differentiation. Further mechanistic investigations are needed to establish if thymosin .beta.4 is differentially expressed in vessels and if it directly induces differentiation in endothelial cells or if it is a product of the differentiation process.

L37 ANSWER 69 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB Thymosin .beta.11 and .beta.12 were isolated from trout spleen and their structures detd. and verified by solid-phase synthesis.

L37 ANSWER 73 OF 153 MEDLINE DUPLICATE 21
AB Thymosin beta 4 containing 43 amino-acid residues belongs to a family of highly homologous peptides present at high concentrations in various species, cells, and tissues. Safer et al. [J. Biol. Chem. 266, 4029-4032 (1991)] have shown that thymosin beta 4 is an actin-sequestering peptide. Because DNase I is inhibited by G-actin and not by F-actin we employed this enzymatic assay to determine the actin sequestering properties of 4 other thymosin beta 4-like peptides and fragments of

thymosin beta 4 generated by enzymatic digestions. Thymosin beta 4 sequesters G-actin at a 1 to 1 ratio an thereby inhibits its polymerisation to F-actin in high salt solution. The oxidation of the single methionine residue at position 6 does not abolish its actin-sequestering properties. However neither thymosin beta 4 24-43 nor thymosin beta 4 13-43 inhibit the polymerisation of G-actin. We conclude from this that some structural features in the amino-acid **sequence** of **thymosin beta** 4 before position 13 are obligatory for its biological function. Oxidized thymosin beta 4 (beta 4-sulfoxide) as well as four other thymosin beta 4-like peptides were shown to be actin-sequestering peptides like thymosin beta 4.

L37 ANSWER 90 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB At least 50% of the actin in resting human platelets is unpolymd., and the bulk of this unpolymd. actin is complexed with a recently identified acidic, heat-stable 5-kDa peptide, named Fx. Purified Fx binds stoichiometrically to muscle G-actin, forming a complex identifiable by nondenaturing polyacrylamide gel electrophoresis. Formation of the complex inhibits salt-induced polymn. of G-actin. Fx is indistinguishable from thymosin .beta.4, an acidic, heat-stable 5-kDa peptide first isolated from calf thymus and thought to be a thymic hormone. The complete amino acid sequence of Fx was detd. and was found to be identical with that of thymosin .beta.4. Authentic thymosin .beta.4 is functionally equiv. to Fx, forming a 1:1 complex with actin monomers and inhibiting polymn. The widespread distribution and high intracellular concn. of thymosin .beta.4 (Fx) strongly suggest that it plays a significant role in regulating actin polymn. in many cell types.

L37 ANSWER 99 OF 153 MEDLINE DUPLICATE 35
AB We have isolated a cDNA encoding the human interferon-inducible gene 6-26, by screening a cDNA library with an oligodeoxynucleotide probe. Its sequence was found to be identical to that of the human thymosin-beta 4 cDNA, which encodes a protein present in most cell types, but whose function is not clear at present. By hybridization of the thymosin-beta 4/6-26 cDNA to the DNA of a panel of human-rodent somatic cell hybrids, we found that at least seven genes homologous to this cDNA are present in the human genome. We localized these genes, some of which might be pseudogenes, to seven distinct chromosomes, namely, chromosomes 1, 2, 4, 9, 11, 20, and X.

L37 ANSWER 118 OF 153 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

L37 ANSWER 129 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB The isolation and chem. characterization of prothymosin .alpha., the precursor of thymosin .alpha.1, and thymosin .beta.4, are described as well as the mol. cloning of their cDNA's. A 3rd peptide, parathymosin, was also isolated and its primary structure detd. Parathymosin is similar in size and amino acid compn. to prothymosin .alpha., and shares some amino acid sequences. Preliminary observations on the biol. properties of prothymosin .alpha. and parathymosin are discussed.

L37 ANSWER 136 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB A review with 23 refs. is given on the purifn., assay, and chem. characterization of thymosin .alpha.1 [69521-94-4] and polypeptide .beta.1 [56574-49-3] from thymosin fraction 5. Amino acid sequences for the 2 peptides are given. Thymosin .alpha.1 was biol. active in the terminal deoxynucleotidyltransferase and macrophage migration inhibitory factor biol. tests, but polyupeptide .beta.1 was inactive.

L37 ANSWER 141 OF 153 HCAPLUS COPYRIGHT 2003 ACS
AB Novel peptides, designated thymosin .beta.8 [81775-03-3] and thymosin .beta.9 [81775-04-4], were isolated from calf thymus and their amino acid sequences established. Thymosin .beta.9 was isolated by homogenizing frozen calf thymus in an ice cold aq. soln. contg. the antiproteolytic

agent guanidine-HCl (6M). The homogenate is suspended in a 0.2M pyridine-1M formic acid soln., centrifuged, filtered successively through Whatman No. 541 and Whatman No. 1 filters, pumped through a hollow fiber concg. system with an H1P10 cartridge, and then adsorbed by a reverse phase RP8 column (40-63 nm). The peptides are then eluted from the column with 40% m-propanol in 0.4M pyridine-0.5M formic acid at pH 4.0. Thymosin .beta.9 is then sepd. from thymosin .beta.4 by high-pressure liq. chromatog. utilizing a reverse phase C18 hydrophobic resin and eluting the resin with a gradient of increasing concns. of m-propanol from 0 to 40% by vol. in 0.2M pyridine-1M formic acid buffer soln. of about pH 4.0. The thymosin .beta.9 yield was 8-18 .mu.g/g tissue. These peptides may be useful in treating infections in immunosuppressed individuals.

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FULL ESTIMATED COST                                      40.37      40.58

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CA SUBSCRIBER PRICE                                      -5.21      -5.21

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 09:45:32 ON 27 JUN 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

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FILE 'HCAPLUS'
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L42 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI **Sequence** homologs of **thymosin** .**beta**.10, ephrin A8 receptors and fibromodulin and cDNA encoding them and theor therapeutic uses
SO PCT Int. Appl., 180 pp.
CODEN: PIXXD2
IN Prayaga, Sudhirdas K.; Taupier, Raymond J.; Bandaru, Raj
AN 2002:293699 HCAPLUS
DN 136:320393

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002030979	A2	20020418	WO 2001-US31498	20011010 <--
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AU 2002011543	A5	20020422	AU 2002-11543	20011010 <--

L42 ANSWER 2 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI Gene markers useful for detecting skin damage in response to ultraviolet radiation
SO PCT Int. Appl., 274 pp.
CODEN: PIXXD2
IN Blumenberg, Miroslav
AN 2002:185378 HCAPLUS
DN 136:212896

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002020849	A2	20020314	WO 2001-US28214	20010907 <--
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AU 2001090699	A5	20020322	AU 2001-90699	20010907 <--

L42 ANSWER 3 OF 10 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Identifying maintenance genes comprises determining expression of 100 genes in different types of tissues at 2 different developmental stages and identifying genes expressed at same level in different tissues; DNA array and DNA probe for human gene expression study
AU Warrington J A; Mahadevappa M; Nair A
AN 2001-09842 BIOTECHDS
PI **WO** 2001029267 26 Apr 2001

L42 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
TI Identification of genes involved in metastasis
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
IN Clark, Edwin A.; Golub, Todd R.; Hynes, Richard O.; Lander, Eric S.
AN 2001:434910 HCAPLUS
DN 135:32378

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001041815	A2	20010614	WO 2000-US33631	20001211 <--
WO 2001041815	A3	20020801		
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US 2001044414	A1	20011122	US 2000-735273	20001211 <--
EP 1274463	A2	20030115	EP 2000-984232	20001211 <--

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L42 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
TI Protein and cDNA sequences of novel human protein NOV1-3 and use thereof
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
IN Prayaga, Sudhirdas K.; Taupier, Raymond J., Jr.; Bandaru, Raj
AN 2001:300870 HCAPLUS
DN 134:336696

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001029217	A2	20010426	WO 2000-US28474	20001013 <--
WO 2001029217	A3	20020110		

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EP 1222272	A2	20020717	EP 2000-973541	20001013 <--

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L42 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI Nucleic acid sequences associated with baldness and uses in detecting the likelihood of baldness and for gene therapy
SO PCT Int. Appl., 87 pp.
CODEN: PIXXD2
IN Pritchard, David; Burmer, Glenna; Brown, Joseph; Demas, Vasiliki
AN 2001:798473 HCAPLUS
DN 135:340282

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001081628	A1	20011101	WO 2001-US12184	20010413 <--
WO 2001081628	C2	20021227		

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US 2002177566	A1	20021128	US 2001-825096	20010402 <--

L42 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI Nucleic acid markers useful for the identification, assessment, prevention and therapy of human cancers
SO PCT Int. Appl., 126 pp.
CODEN: PIXXD2
IN Roth, Frederick P.; Van Huffel, Christophe; White, James V.; Shyjan, Andrew W.
AN 2001:618207 HCAPLUS

DN 135:190398

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001061048	A2	20010823	WO 2001-US5263	20010216 <--
WO 2001061048	A3	20030123		

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US 2002051978	A1	20020502	US 2001-788100	20010216 <--

L42 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI Methods for treatment of human Huntington's disease and methods of screening for active agents
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
IN Olson, James M.; Luthi-Carter, Ruth; Young, Anne; Strand, Andrew
AN 2001:360024 HCAPLUS
DN 134:361383

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001034633	A2	20010517	WO 2000-US30900	20001110 <--
WO 2001034633	A3	20020110		

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AU 2001017602	A5	20010606	AU 2001-17602	20001110 <--

L42 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI Methods of determining individual hypersensitivity to a pharmaceutical agent from gene expression profile
SO PCT Int. Appl., 222 pp.
CODEN: PIXXD2
IN Farr, Spencer
AN 2001:338762 HCAPLUS
DN 134:362292

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001032928	A2	20010510	WO 2000-US30474	20001103
WO 2001032928	A3	20020725		

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L42 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2003 ACS
TI Nucleic acids and proteins associated with cancer as antitumor targets
SO PCT Int. Appl., 98 pp.
CODEN: PIXXD2
IN Burmer, Glenna C.; Brown, Joseph P.; Pritchard, David
AN 2001:320060 HCAPLUS
DN 134:339179

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

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PI   WO 2001030964     A2   20010503       WO 2000-US29126  20001020 <--
     WO 2001030964     A3   20010809
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             HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
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     AU 2001013397     A5   20010508       AU 2001-13397    20001020 <--


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3	82	thymosin adj beta	USPAT; US-PGPUB	2003/06/26 16:16
4	16	(thymosin near5 (gene$1 or sequence$1)) and (thymosin adj beta)	USPAT; US-PGPUB	2003/06/26 16:16

PGPUB-DOCUMENT-NUMBER: 20030099617

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030099617 A1

TITLE: Method for using **thymosin beta-10 for gene** therapy of solid malignant tumors

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lee, Je-Ho	Seoul		KR	
Kim, Seung-Hoon	Seoul		KR	

APPL-NO: 10/ 231845

DATE FILED: August 30, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
KR	2001-63524	2001KR-2001-63524	October 10, 2001

US-CL-CURRENT: 424/93.2, 435/235.1 , 435/320.1 , 435/456

ABSTRACT:

A method for using **thymosin .beta.**-10 for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumor cells. More precisely, the present invention relates to a cancer treatment method wherein **thymosin .beta.**-10 is expressed in solid malignant tumor cells by infecting adenovirus including **thymosin .beta.**-10. The **gene therapy for cancer using thymosin .beta.**-10 of the present invention is very effective for the treatment of ovarian cancer, cervical cancer, stomach cancer and lung cancer.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

A method for using **thymosin .beta.**-10 for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumor cells. More precisely, the present invention relates to a cancer treatment method wherein **thymosin .beta.**-10 is expressed in solid malignant tumor cells by infecting adenovirus including **thymosin .beta.**-10. The **gene therapy for cancer using thymosin .beta.**-10 of the present invention is very effective for the treatment of ovarian cancer, cervical cancer, stomach cancer and lung cancer.

Title - TTL (1):
Method for using **thymosin beta-10 for gene** therapy of solid malignant tumors

Summary of Invention Paragraph - BSTX (4):
[0002] The present invention relates to a method for using **thymosin .beta.**-10 for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumor cells. More precisely, the present invention relates to a cancer treatment method wherein **thymosin .beta.**-10 is expressed in solid malignant tumor cells by infecting adenovirus including **thymosin .beta.**-10. The **gene therapy for cancer using thymosin .beta.**-10 of the present invention is very effective for the treatment of ovarian cancer, cervical cancer, stomach cancer and lung cancer.

Summary of Invention Paragraph - BSTX (9):
[0006] **Thymosin .beta.**-4, .beta.-10 and .beta.-15 act as major actin monomer-sequestering factors. **Thymosin .beta.**-4 has 43 amino acids and shares a high degree of homology(85%) at the amino acid level with **thymosin .beta.**-10. A number of investigations have now suggested that the role of **thymosin .beta.**-4 and .beta.-10 may be related to mechanisms associated with cell division and/or differentiation. Despite these gene's structural and functional similarities, different expression patterns have been observed. For example, while both thymosins were strongly expressed in fetal brain and other fetal organs, **thymosin .beta.**-10 levels fell considerably in most adult tissues, and **thymosin .beta.**-4 expression was down-regulated in metastatic cells of colorectal carcinomas(Hall et al., Mol. Brain Res., 1990, 8:129-135; Hall et al., Mol. Cell. Endocrinol., 1991, 79:37-41; Yamamoto et al., Biochem. Biophys. Res. Commun., 1993, 193:706-710). Another recently discovered member of the .beta.-thymosin family, **thymosin .beta.**-15, is upregulated in aggressive human prostate cancer (Bao et al., Nat. Med., 1996, 2:1322-28). It is expressed in highly motile, metastatic prostate cancer cells as well as in advanced human prostate and breast cancer (Eadie et al., J. Cell, Biochem., 2000, 77:277-287; Gold et al., Mod. Pathol., 1997, 10:1106-12). **Thymosin .beta.**-15 differs from other .beta.-thymosins in that its expression correlates with motility and metastasis in highly metastatic prostate carcinoma cells.

Summary of Invention Paragraph - BSTX (10):
[0007] **Thymosin .beta.**-10 is a small actin-binding protein known to sequester actin monomers and thereby induce depolymerization of the intracellular F-actin networks (Nachmias, Curr. Opin. Cell Biol., 1993, 5:56-62; Yu et al., J. Biol Chem., 1993, 268:502-9; Yu et al., Cell Motil. Cytoskeleton, 1994, 27:13-25). Actin is one of the most abundant structural proteins in the cell (Pollard and Cooper, Ann. Rev. Biochem., 1986, 55:987-1035), and the dynamic equilibrium between monomeric and filamentous actin is shown to be altered in neoplastic/transformed cells (Hall, Ren Fail., 1994, 16:243-54). Alteration of **thymosin .beta.**-10 expression may thus affect the cellular infrastructure by changing the actin stress fiber, which may further alter the balance of cell growth, cell death, cell attachment and cell migration (Yu et al., J. Biol Chem., 1993, 268:502-9). During embryogenesis,

thym sin .beta.-10 is also highly expressed (Carpintero et al., FEBS Lett., 1996, 394:103-6), which is consistent with constant cell migration and morphogenesis that require cell detachment. **Thymosin .beta.**-10 was also shown to be involved in inducing processes leading to cell detachment (Iguchi et al., Eur. J. Biochem., 1998, 253:766-770). **Thymosin .beta.**-10 has also been proposed to have dual functions: programmed cell death and invasion or metastasis (Hall, Cell. Mol. Biol. Res., 1995, 41:167-180; Marian et al., Int. J. Cancer, 1993, 53:278-84).

Summary of Invention Paragraph - BSTX (12):

[0009] In order to identify proper genes useful for gene therapy for solid malignant tumors, the present inventors searched abnormally expressed genes in solid malignant tumor cells, comparing to normal cell tissues and at last discovered that the expression of **thymosin .beta.**-10 is decreased remarkably in ovarian cancer cell tissues, compared to normal ovarian cells. And finally, the present inventors have accomplished the present invention by discovering that **thymosin .beta.-10 could be used for gene** therapy for ovarian cancer, cervical cancer and lung cancer, since **thymosin .beta.**-10 expressed in adenovirus could suppress the solid malignant tumor cell growth or induce apoptosis of tumor cells.

Summary of Invention Paragraph - BSTX (14):

[0010] It is an object of this invention to provide a method for using **thymosin .beta.**-10 for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumor cells.

Summary of Invention Paragraph - BSTX (15):

[0011] To accomplish the object, the present invention provides a method for using **thymosin .beta.**-10 for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumor cells. The method comprises introducing an exogenous **thymosin .beta.-10 gene** into solid malignant tumor cells to obtain expression of **thymosin .beta.**-10 in the tumor cells.

Summary of Invention Paragraph - BSTX (16):

[0012] The present invention also provides an adenovirus expression vector containing **thymosin .beta.-10 gene**.

Summary of Invention Paragraph - BSTX (18):

[0014] The present invention also provides a method for using the above adenovirus for the treatment of solid malignant tumors. The invention additionally provides a method for inducing apoptosis, inhibiting growth of cancer cells, and/or disrupting F-actin stress fibers in **thymosin .beta.**-10-deficient cancer cells. The method comprises contacting the **thymosin .beta.**-10-deficient cancer cells with an expression vector containing an exogenous **thymosin .beta.-10 gene**.

Summary of Invention Paragraph - BSTX (19):
[0015] In a preferred embodiment, the contacting comprises infecting the cancer cells with an adenovirus modified to produce **thym sin .beta.**-10. The infecting can be by natural viral entry into the cells or by transfection, as is understood by those skilled in the art.

Brief Description of Drawings Paragraph - DRTX (6):
[0020] a: Smad 1, b: **Thymosin .beta.**-10

Brief Description of Drawings Paragraph - DRTX (9):
[0023] FIG. 2A is a photograph showing the result of Northern blot analysis of **thymosin .beta.**-10 expression;

Brief Description of Drawings Paragraph - DRTX (12):
[0026] FIG. 2B is a photograph showing the result of PCR analysis of **thymosin .beta.**-10 expression;

Brief Description of Drawings Paragraph - DRTX (15):
[0029] FIG. 2C is a photograph showing the result of PCR analysis of **thymosin .beta.**-10 expression (Human ribosomal protein S9 was used as control);

Brief Description of Drawings Paragraph - DRTX (18):
[0032] FIG. 3 is a schematic diagram showing the genetic map of adenovirus expression vector containing the **thymosin .beta.-10 gene**;

Brief Description of Drawings Paragraph - DRTX (19):
[0033] FIG. 4 is a photograph showing the result of Western blot analysis of **thymosin .beta.**-10 expression in infected PA-1 ovarian cancer cell lines;

Brief Description of Drawings Paragraph - DRTX (20):
[0034] FIG. 5 is graphs showing the effect of **thymosin .beta.**-10 on the growth of ovarian cancer cell lines PA-1 and SKOV3;

Brief Description of Drawings Paragraph - DRTX (22):
[0036] .smallcircle.: Control, .circle-solid.: Ad-GFP, .box-solid.: Ad-GFP-**thymosin .beta.**-10

Brief Description of Drawings Paragraph - DRTX (23):
[0037] FIG. 6 is microphotographs showing the results analysis of apoptotic cell death induced by Av-GFP or Av-GFP-**thymosin .beta.**-10 transfection in ovarian cancer cell line PA-1. Cells were stained with DAPI, and GFP expression was examined by fluorescence microscopy;

Brief Description of Drawings Paragraph - DRTX (25):
[0039] E,F: Av-GFP-**thymosin .beta.**-10

Brief Description of Drawings Paragraph - DRTX (26):
[0040] FIG. 7 is microphotographs showing the results of phalloidin-FITC staining assay, which shows structural changes of actin induced by Av-GFP or Av-GFP-**thymosin .beta.**-10 transfection in ovarian cancer cell line PA-1;

Brief Description of Drawings Paragraph - DRTX (27):
[0041] A: Control, B: Av-GFP, C: Av-GFP-**thymosin .beta.**-10

Detail Description Paragraph - DETX (2):
[0043] In one aspect, the present invention provides a method for using **thymosin .beta.**-10 for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumor cells.

Detail Description Paragraph - DETX (3):
[0044] In the preferred embodiments, **thymosin .beta.**-10 was overexpressed in solid malignant tumor cells to suppress tumor cell growth, induce apoptosis and kill the tumor cells.

Detail Description Paragraph - DETX (4):
[0045] **Thymosin .beta.**-10 is expressed at much higher levels in ovarian tissue than in pancreatic, thymus, prostate, testicular and colon tissues. And the expression is decreased in ovarian cancer tissue compared with normal ovarian tissue (see FIG. 2). **Thymosin .beta.**-10 is a small actin-binding protein known to sequester actin monomers and thereby induce depolymerization of the intracellular F-actin network. Thus, alteration of **thymosin .beta.**-10 expression may affect the cellular infrastructure by changing the actin stress fiber, which may further alter the balance of cell growth, cell death, etc.

Detail Description Paragraph - DETX (5):
[0046] In the preferred embodiments of the present invention, **thymosin .beta.**-10 whose expression was specifically decreased in solid malignant tumor tissues was expressed in solid malignant tumor cells. **Thymosin .beta.**-10 expressed in solid malignant tumor cells causes the alteration of actin stress fibers, leading to the suppress of solid malignant tumor cell growth or even to the death of those cancer cells.

Detail Description Paragraph - DETX (6):
[0047] In the preferred embodiments of the present invention, **thymosin .beta.**-10 was used for cancer treatment by expressing **thymosin .beta.**-10 in solid malignant tumors such as ovarian cancer, cervical cancer, stomach cancer, lung cancer and liver cancer cells.

Detail Description Paragraph - DETX (7):
[0048] The present invention also provides a adenovirus expression vector containing **thymosin .beta.-10 gene**.

Detail Description Paragraph - DETX (8):
[0049] The present invention provides a adenovirus expression vector which can produce **thymosin .beta.**-10 protein by using expression cassette consisting of coding regions for promoter site and multiple cloning site of cytomegalovirus (CMV), late polyadenylation signal site of simian virus 40 (SV 40) and green fluorescence protein (GFP).

Detail Description Paragraph - DETX (10):
[0051] In the present invention, pQBI-Ad5CMV-GFP vector containing expression cassette consisting of coding regions for promoter site and multiple cloning site of cytomegalovirus (CMV), late polyadenylation signal site of simian virus 40 (SV 40) and green fluorescence protein (GFP) was used. In order to separate **thymosin .beta.-10 gene**, RT-PCR was performed with primers represented by the SEQ. ID. NO: 1 and 2. At this time, RNA purified from normal tissues was used as a template. Finally, normal human **thymosin .beta.**-10 cDNA was obtained. The above **thymosin .beta.**-10 cDNA was inserted into multiple cloning site of pQBI-Ad5CMV-GFP vector, and then Ad-GFP-**thymosin .beta.**-10 was constructed. The above adenovirus expression vector "Ad-GFP-**thymosin .beta.-10" was deposited at Gene** Bank of Korea Research Institute for Bioscience and Biotechnology on Oct. 8, 2001 (Accession No: KCTC 10089BP).

Detail Description Paragraph - DETX (13):
[0054] The present invention provides a adenovirus clone without RCV selected from adenovirus proliferation by injecting adenovirus expression vector Ad-GFP-**thymosin .beta.**-10 into packaging cell line 293 along with adenovirus mother vector.

Detail Description Paragraph - DETX (15):
[0056] In the preferred embodiment of the present invention, the present inventors have infected ovarian cancer cells with Ad-GFP-**thymosin .beta.**-10 in order to detect its effect on ovarian cancer cell growth. As a result, Ad-GFP-**thymosin .beta.**-10 infected cells showed decreased cell-growth comparing to uninfected or normal cells (see FIG. 5).

Detail Description Paragraph - DETX (16):
[0057] The present inventors have infected ovarian cancer cells with Ad-GFP-**thymosin .beta.**-10 in order to investigate its effect on apoptosis of ovarian cancer cells. As a result, apoptosis of Ad-GFP-**thymosin .beta.**-10 infected cells was rapidly increased (see FIG. 6), and F-actin stress fibers of infected cells were disrupted because of overexpression of **thymosin .beta.**-10

(see FIG. 7). As explained above, **thym sin .beta.**-10 expressed in solid malignant tumor cells cause the alteration of actin stress fibers, leading to the suppress of solid malignant tumor cell growth or even to the death of those cancer cells.

Detail Description Paragraph - DETX (34):

[0072] As shown in Tables 1 and 2, six genes showed increased expression in ovarian cancer tissues: Smad1 (Mothers against DPP protein), prothymosin alpha, Tob, C-1, heat shock 27-kDa protein 1, and insulin-like growth factor (Table 1). The expression of 12 genes was decreased in ovarian cancer tissues, including a group of apoptosis-related proteins, DNA-binding proteins, DNA-binding protein inhibitors, transcription factors and **thymosin .beta.**-10(Table 2). Among them, **thymosin .beta.**-10 showed consistently decreased expression levels in four of five cancer samples.

Detail Description Paragraph - DETX (36):

Analysis of **Thymosin .beta.**-10 Expression Pattern

Detail Description Paragraph - DETX (37):

[0073] In order to investigate the expression pattern of **thymosin .beta.**-10 showing decreased expression levels in ovarian cancer tissues, the present inventors performed Northern blot and PCR.

Detail Description Paragraph - DETX (39):

[0075] For Northern blot hybridization, total RNA extracted from five pairs of normal and ovarian cancer tissues in the above example <1-1> was used. Total RNA(10 .mu.g) was denatured in the presence of 50% formamide, 2.2 M formaldehyde, 20 mM MOPS(3-[N-morpholino] propanesulfonic acid), 4 mM sodium acetate and 0.5 mM EDTA at 65.degree. C. for 10 minutes. After electrophoresis in a 1.2% agarose gel containing 2.2 M formaldehyde, RNA was transferred onto a nylon membrane (Nytran, 0.45-.mu.m pore size; Schleicher and Schuell, Germany) by capillary action under 10.times.SSPE (1.times.SSPE; 0.18 M NaCl, 10 mM Na.sub.2HPO.sub.4 [pH 7.7], 1 mM EDTA). RNA transfer and loading efficiency was estimated by staining a separate membrane with 0.1% methylene blue. RNA intactness was estimated by comparing the intensities of the 28S and 18S ribosomal RNA bands. For hybridization, the membrane was washed in 6.times.SSPE for 5 minutes and air-dried, and the RNA was permanently attached to the membrane by 1 minute UV illumination. Hybridization was performed overnight in a heat-sealable polyethylene bag containing 40 Ml of hybridization buffer (5.times.SSPE[PH 7.4], 5.times. Denhardt's solution, 0.5% SDS, 0.2 mg/Ml heat-denatured salmon sperm DNA, 50% formamide) and the hybridization probe. The **thymosin .beta.**-10 cDNA(178 bp) probe containing the entire coding sequence was obtained by PCR amplification with the primers represented by the SEQ. ID. NO: 1 and 2. .sup.32P-labeled cDNA probes were synthesized using a Rediprime cDNA synthesis kit (Amersham).

Detail Description Paragraph - DETX (40):

[0076] As a result, Northern blot analysis confirmed decreased **thymosin**

.beta.-10 mRNA levels in four of five ovarian tumors(FIG. 2A). In FIG. 2A, the numbers below each T lane represent the ratio of hybridization signals in cancer/normal tissues.

Detail Description Paragraph - DETX (42):

[0078] In order to confirm the differential expression of **thymosin .beta.**-10, the present inventors performed PCR analysis of **thymosin .beta.**-10 with two matched human ovarian cDNA pairs(tumor versus normal; Clontech Laboratories, Palo Alto, Calif., USA) as templates. The first fair was the cDNA of ovary serous cystadenocarcinoma and normal tissue, and the second fair was the cDNA of ovary papillary serous carcinoma and normal tissue. PCR was performed at the following cycles: 30 seconds at 94.degree. C.; 30 cycles of 30 seconds at 94.degree. C.; 1 minute at 68.degree. C.; 1 minute at 72.degree. C.; 5 minutes at 72.degree. C., according to the manufacturer's protocols (Clontech). The present inventors used primers represented by the SEQ. ID. NO: 3 and 4 for **thymosin .beta.**-1, and we used human ribosomal protein S9 primers represented by the SEQ. ID. NO: 5 and 6 as control.

Detail Description Paragraph - DETX (43):

[0079] As a result, **thymosin .beta.**-10 expression was decreased in cancer cells in one of the two pairs (FIG. 2B).

Detail Description Paragraph - DETX (44):

[0080] <2-3> **Thymosin .beta.**-10 Expression in Normal Ovary and Other Organs

Detail Description Paragraph - DETX (45):

[0081] In order to confirm the expression pattern of **thymosin .beta.**-10 in various normal tissues, total RNA was isolated from normal spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes, and Northern blot analysis was performed with the total RNA by the same method as in Example <2-1>.

Detail Description Paragraph - DETX (46):

[0082] As a result, **thymosin .beta.**-10 expression was found to be highest in normal ovary compared with other organs examined, including spleen, thymus, prostate, testis, small intestine, colon and peripheral blood leukocytes(FIG. 2C). From the above results, it was suggested that **thymosin .beta.**-10 is downregulated in human ovarian cancer and may play an important role in human ovarian carcinogenesis.

Detail Description Paragraph - DETX (48):

Analysis of **Thymosin .beta.**-10 Expression Pattern by SAGE

Detail Description Paragraph - DETX (49):

[0083] In order to confirm the expression pattern of **thymosin .beta.**-10 in ovarian cancer versus normal cells, the present inventors analyzed the data of its expression in the ovarian SAGE(serial analysis of gene expression) libraries available at the NCBI database (Hough, et al., Cancer Res., 2000, 60:6281-87).

Detail Description Paragraph - DETX (50):

[0084] For SAGE analysis, the frequencies of tags represent the relative expression of the genes in the cell population. As shown in Table 3, similar frequencies for **thymosin .beta.**-10 were seen in a normal ovarian epithelial cell line, an SV40 large T antigen-transformed ovarian epithelial cell line, and three of ten tumors, whereas lower frequencies were found in six of the 10 tumors; the frequency of **thymosin .beta.**-10 expression was increased only in one case of them.

Detail Description Paragraph - DETX (51):

[0085] From the above results, it was suggested that **thymosin .beta.**-10 is downregulated in approximately 60% of ovarian cancers and may play an important role in the development of this subset of ovarian cancers.

Detail Description Paragraph - DETX (53):

Construction of Adenovirus Expression Vector Containing **Thymosin .beta.**-10

Detail Description Paragraph - DETX (54):

[0086] In order to construct adenovirus expression vector containing **thymosin .beta.**-10, firstly, **thymosin .beta.-10 gene** was isolated. Particularly, full-length human **thymosin .beta.**-10 (349 bp) was amplified by using the primers represented by the SEQ. ID. NO: 7 and 8. The amplified human **thymosin .beta.**-10 fragment was cloned into pCRII-TOPO vector (Invitrogen).

Detail Description Paragraph - DETX (55):

[0087] The cloning product was confirmed for sequence from both directions, and named as "pCR-TOPO-**thymosin .beta.**-10".

Detail Description Paragraph - DETX (56):

[0088] The pCR-TOPO-**thymosin .beta.**-10 was digested with EcoRI and cloned into the p.DELTA.ACMV EcoRI site. The BamHI fragment of **thymosin .beta.**-10 was inserted into the BglII site of pQBI-Ad5CMV-GFP vector(Quantum, Canada), and it was named as "Ad-GFP-**thymosin .beta.**-10" (FIG. 3). pQBI-Ad5CMV-GFP vector having 9.4-15.5 map units has been used as a shuttle vector of adenovirus backbone vector pJM17, and GFP (Green Fluorescence Protein) of the vector makes it easy to analyze gene transmission under the condition of virus infection.

Detail Description Paragraph - DETX (57):

[0089] The above-mentioned expression vector "Ad-GFP-thymosin .beta.-10" of the present invention was deposited at Gene Bank of Korea Research Institute of Bioscience and Biotechnology on Oct. 8, 2001 (Accession No.: KCTC 10089BP).

Detail Description Paragraph - DETX (59):
Construction of Adenovirus Clone Without RCV Which can Produce Thymosin .beta.-10 Proteins Within Cells

Detail Description Paragraph - DETX (60):
[0090] In order to construct adenovirus clone which can produce thymosin .beta.-10 proteins by infecting thereof into cells, the Ad-GFP-thymosin .beta.-10 and a adenovirus mother vector pJM17 (McGrory, et al., Virology, 1988, 163, 614-617) were cotransfected into a packaging cell line, 293 cells by phosphate-calcium method.

Detail Description Paragraph - DETX (65):
Infection of Ad-GFP-Thymosin .beta.-10 into Human Ovarian Cancer Cells

Detail Description Paragraph - DETX (67):
[0095] The present inventors performed Western blot analysis to confirm whether the thymosin .beta.-10 was expressed in cells infected with the adenovirus. Particularly, cells were lysed and equal amount of cell extracts(10 .mu.g) were electrophoresed on 15% SDS polyacrylamide gel, electrotransferred onto a nitrocellulose membrane, and probed with rabbit anti-thymosin .beta.-10 antibody(provided by Dr Leondiadis L, Institute of Radioisotopes and Radiodiagnostic Products, NCSR Demokritos, Athens, Greece). Thymosin .beta.-10 expression was detected using the enhanced chemiluminescence system (ECL, Amersham).

Detail Description Paragraph - DETX (68):
[0096] As a result, strong expression of thymosin .beta.-10 in the cells infected with adenovirus of the present invention was observed(FIG. 4).

Detail Description Paragraph - DETX (70):
Effect of Thymosin .beta.-10 on Ovarian Cancer Cell Growth

Detail Description Paragraph - DETX (71):
[0097] The present inventors infected ovarian cancer cell lines with adenovirus prepared in the above Example 5 and observed growth of the cells to investigate the effect of thymosin .beta.-10 on the ovarian cancer cell growth. Particularly, ovarian cancer cells (PA-1 and SKOV3) were plated in triplicate at a density of 2.times.10.sup.5 cells/well in 6-well plate. Twenty-four hours later, the cells were infected with Ad-GFP-thymosin .beta.-10. Beginning 24 h after infection, cells were harvested each day, stained with Trypan blue and counted with the light microscope for up to 3 days (PA-1) or up to 5 days

(SKOV3).

Detail Description Paragraph - DETX (74):
Effect of **Thymosin .beta.**-10 on Apoptosis of Ovarian Cancer Cells

Detail Description Paragraph - DETX (75):
[0099] The present inventors infected ovarian cancer cell lines with adenovirus prepared in the above Example 5 and observed apoptosis of the cells to investigate the effect of **thymosin .beta.**-10 on the apoptosis of ovarian cancer cells. Particularly, an ovarian cancer cell line, PA-1 cells were plated onto 4-chamber slides (Nalgen Nunc, Inc., Naperville, Ill., USA) at a density of 5.times.10.sup.4 cells/well and cultured for 1 day. Two days after infection with Ad-GFP-**thymosin .beta.**-10 and Ad-GFP, chamber slides were rinsed with phosphate buffered saline(PBS), stained with 2 mg/Ml of DAPI (4,6-diamidino-2-phenylindole, Boehringer Mannheim) at 37.degree. C. for 15 minutes, washed twice with PBS and examined with fluorescence microscope.

Detail Description Paragraph - DETX (76):
[0100] As a result, PA-1 cells infected with adenovirus of the present invention showed clear DNA fragmentation, suggesting that overexpression of **thymosin .beta.**-10 induced massive cell death (FIG. 6).

Detail Description Paragraph - DETX (78):
Effect of **Thymosin .beta.**-10 on Actin Structure of Ovarian Cancer Cells

Detail Description Paragraph - DETX (79):
[0101] Because **thymosin .beta.**-10 is an actin-binding protein, the present inventors reasoned that **thymosin .beta.**-10 expression might act by altering actin stress fibers in the cells.

Detail Description Paragraph - DETX (80):
[0102] Ovarian cancer cell line PA-1 was infected with adenovirus (Ad-GFP-**thymosin .beta.**-10) of the present invention, and the cell monolayers were fixed with 4% paraformaldehyde in PBS for 40 minutes at room temperature and then stained with 25 .mu.g/Ml of phalloidin-FITC (Sigma) in the dark for 1 hour. Stained cell monolayers were washed twice with 0.5% triton X-100 in PBS. Coverslips were mounted onto slides using a PBS/glycine mountant and examined with the fluorescence microscope.

Detail Description Paragraph - DETX (81):
[0103] As a result, PA-1 cells infected with mock or Ad-GFP were confirmed to have intact and pervasive actin structure, but the F-actin stress fibers in the cells infected with Ad-GFP-**thymosin .beta.**-10 were disrupted, suggesting that overexpression of **thymosin .beta.**-10 induced degradation of F-actin stress fibers (FIG. 7).

Detail Description Paragraph - DETX (82):

[0104] As shown above, a cancer treatment method in which **thymosin .beta.**-10 is expressed in solid malignant tumor cells by infecting adenovirus including **thymosin .beta.**-10 of the present invention can be effectively used for gene therapy for the treatment of ovarian cancer, cervical cancer, stomach cancer, lung cancer and liver cancer.

Detail Description Table CWU - DETL (2):

2 TABLE 2 Case Case Case Case Case Position Name of protein/Gene 1 2 3 4 5 A1c MYB proto-oncogene - - NC NC NC protein B4n Tyrosine-protein kinase - - NC NC NC JAK1 C1c Tumor necrosis factor - NC NC - NC receptor C2d Tyrosine kinase ligand - - - NC NC C5e HDLC1 (cytoplasmic NC - - NC NC dynein light chain 1) D1d DNA binding protein NC - - NC - inhibitor ID-3 D1g DNA binding protein NC NC - - - inhibitor ID-2 D3k Guanine nucleotide- NC - NC - - binding protein G-S (alpha subunit) D5h DNA binding protein NC - - - NC SATB1 D5k Transcription factor NC NC - - - PAX3/FORKHEAD D7c Proliferation-associated - - NC - NC protein PAG F4d **Thymosin beta**-10 - - - NC - -: Expression decreased, NC: Not changed, Case 1: Papillary serous adenocarcinoma (stage IIIc), Case 2: Borderline mucinous ovarian tumor (stage Ic), Case 3: Serous cystadenocarcinoma (stage IIIc), Case 4, 5: Clear-cell carcinomas (stage Ic).

Claims Text - CLTX (2):

1. A method for treating cancer, the method comprising introducing an exogenous **thymosin .beta.-10 gene** into solid malignant tumor cells.

Claims Text - CLTX (3):

2. The method as set forth in claim 1, wherein the exogenous **thymosin .beta.-10 gene** is introduced by infecting the solid malignant tumor cells with an adenovirus modified to express an exogenous **thymosin .beta.-10 gene**.

Claims Text - CLTX (4):

3. An adenovirus expression vector containing a **thymosin .beta.-10 gene**.

Claims Text - CLTX (7):

6. An adenovirus expression vector that produces **thymosin .beta.**-10 protein in cells.

Claims Text - CLTX (9):

8. The adenovirus expression vector as set forth in claim 7, wherein the adenovirus expression vector is Ad-GFP-**thymosin .beta.-10 in which a gene coding thymosin .beta.**-10 protein is introduced in multiple cloning sites (Accession Number: KCTC 10089BP).

Claims Text - CLTX (11):

10. The adenovirus as set forth in claim 9, wherein the adenovirus expression vector is Ad-GFP-**thymosin .beta.**-10.

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ABSTRACT:

Disclosed herein are nucleic acid sequences that encode G-coupled protein-receptor related polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies, which immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel human nucleic acids and proteins.

RELATED APPLICATIONS

[0001] This application claims priority from U.S. Ser. No. 60/200,158, filed Apr. 26, 2000; U.S. Ser. No. 60/200,613, filed Apr. 28, 2000; U.S. Ser. No. 60/200,780, filed Apr. 28, 2000; U.S. Ser. No. 60/201,006, filed May 1, 2000; U.S. Ser. No. 60/201,007, filed May 1, 2000; U.S. Ser. No. 60/201,236, filed May 1, 2000; U.S. Ser. No. 60/201,238, filed May 1, 2000; U.S. Ser. No. 60/201,186, filed May 2, 2000; U.S. Ser. No. 60/201,474, filed May 3, 2000; U.S. Ser. No. 60/201,508, filed on May 3, 2001; U.S. Ser. No. 60/220,591, filed on Jul. 25, 2000; U.S. Ser. No. 60/232,678 filed Sep. 15, 2000; U.S. Ser. No. 60/263,217 filed Jan. 22, 2001; U.S. Ser. No. 60/265,160, filed Jan. 30, 2001; and Express Mail No. EL585234435US, filed Feb. 16, 2001; each of which is incorporated by reference in its entirety.

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Detail Description Paragraph - DETX (93):
[0115] The full MOL5 amino acid sequence has 37 of 45 amino acid residues (82%) identical to, and 38 of 45 residues (84%) positive with, the 45 amino acid residue **Thymosin beta** protein from Homo sapiens (ptnr: PIR-ID:JC5274) (E=1.2e.sup.-11).

Detail Description Paragraph - DETX (96):

[0118] **Thymosin-beta**-4 induces the expression of terminal deoxynucleotidyl transferase activity in vivo and in vitro, inhibits the migration of macrophages, and stimulates the secretion of hypothalamic luteinizing hormone-releasing hormone. It was noted that the protein was originally isolated from a partially purified extract of calf thymus, thymosin fraction 5, which induced differentiation of T cells and was partially effective in some immuno-compromised animals. Further studies demonstrated that the molecule is ubiquitous; it had been found in all tissues and cell lines analyzed. It is found in highest concentrations in spleen, thymus, lung, and peritoneal macrophages. It was stated that **thymosin-beta**-4 is an actin monomer sequestering protein that may have a critical role in modulating the dynamics of actin polymerization and depolymerization in nonmuscle cells. Its regulatory role is consistent with the many examples of transcriptional regulation of T-beta-4 and of tissue-specific expression. Lymphocytes have a unique T-beta-4 transcript relative to the ubiquitous transcript found in many other tissues and cells. It was stated that rat **thymosin-beta**-4 is synthesized as a 44-amino acid propeptide which is processed into a 43-amino acid peptide by removal of the first methionyl residue. The molecule does not have a signal peptide. Human **thymosin-beta**-4 has a high degree of homology to rat **thymosin-beta**-4; the coding regions differ by only 9 nucleotides, and these are all silent base changes.

Detail Description Paragraph - DETX (97):

[0119] By differential screening of a cDNA library prepared from leukocytes of an acute lymphocytic leukemia patient, a cDNA encoding **thymosin-beta**-4 was isolated. Using Northern blot analysis, the expression of the 830-nucleotide **thymosin-beta**-4 mRNA in various primary myeloid and lymphoid malignant cell lines and in hemopoietic cell lines was studied. It was stated that the pattern of **thymosin-beta-4 gene** expression suggests that it may be involved in an early phase of the host defense mechanism.

Detail Description Paragraph - DETX (98):

[0120] A cDNA clone for the human interferon-inducible gene 6-26 was isolated and showed that its **sequence was identical to that for the human thymosin-beta-4 gene**. By use of a panel of human rodent somatic cell hybrids, it was shown that the 6-26 cDNA recognized seven genes, members of a multigene family, present on chromosomes 1, 2, 4, 9, 11, 20, and X. These genes are symbolized TMSL1, TMSL2, etc., respectively. Li et al. (1996) established that in the mouse there is a single Tmsb4 gene and that the lymphoid-specific transcript is generated by extending the ubiquitous exon 1 with an alternate downstream splice site. By interspecific backcross mapping, they located the mouse gene, which they symbolized Ptmb4, to the distal region of the mouse X chromosome, linked to Btk and Gja6. Thus, the human gene could be predicted to reside on the X chromosome in the general region of Xq21.3-q22, where BTK is located. By analysis of somatic cell hybrids, the **thymosin-beta-4, or TB4X, gene** were mapped to the X chromosome. They noted that a homologous gene, TB4Y, is present on the Y chromosome.

Detail Description Paragraph - DETX (99):

[0121] It was stated that prostate carcinoma is the most prevalent form of cancer in males and the second leading cause of cancer death among older males. The use of the serum prostate-specific antigen test permits early detection of human prostate cancer; however, early detection has not been accompanied by an improvement in determining which tumors may progress to the metastatic stage. The process of tumor metastasis is a multistage event involving local invasion and destruction of extracellular matrix; intravasation into blood vessels, lymphatics or other channels of transport; survival in the circulation; extravasation out of the vessels into the secondary site; and growth in the new location. Common to many components of the metastatic process is the requirement for tumor cell motility. A well-characterized series of cell lines that showed varying metastatic potential was developed from the Dunning rat prostate carcinoma. A direct correlation between cell motility and metastatic potential in the Dunning cell lines was shown. In studies comparing gene expression in poorly and highly motile metastatic cell lines derived from Dunning rat prostate carcinoma using differential mRNA display, Bao et al. (1996) found a novel member of the **thymosin-beta** family of actin-binding molecules. The molecule, named **thymosin-beta**-15 by them, was found to deregulate motility in prostate cells directly. In addition, it was expressed in advanced human prostate cancer specimens, but not in normal human prostate or benign prostatic hyperplasia, suggesting its potential use as a new marker for prostate carcinoma progression. Bao et al. (1996) found that **thymosin-beta**-15 levels correlated positively with the Gleason tumor grade. Coffey (1996) pointed out that the upregulation of **thymosin-beta**-15 as a positive motility factor and the down regulation of the motility suppressor KAI1 (OMIM-600623) provide the `yin and yang` for metastasis; he speculated that these pathways may provide a new target for therapy.

Detail Description Paragraph - DETX (103):

[0125] The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in cancer including but not limited to prostate cancer, immunological and autoimmune disorders (i.e., hyperthyroidism), angiogenesis and wound healing, modulation of apoptosis, neurodegenerative and neuropsychiatric disorders, age-related disorders, and other pathological disorders involving spleen, thymus, lung, and peritoneal macrophages and/or other pathologies and disorders. For example, a cDNA encoding the Beta **Thymosin-like protein may be useful in gene therapy, and the Beta Thymosin**-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from cancer including but not limited to prostate cancer, immunological and autoimmune disorders (i.e., hyperthyroidism), angiogenesis and wound healing, modulation of apoptosis, neurodegenerative and neuropsychiatric disorders, age-related disorders, and other pathological disorders involving spleen, thymus, lung, and peritoneal macrophages. The novel nucleic acid encoding Beta Thymosin-like protein, and the Beta Thymosin-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind

immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

Detail Description Table CWU - DETL (15):

15TABLE 5C BLAST results for MOL5 Gene Index/ Length Identity Positives Identifier Protein/ Organism (aa) (%) (%) Expect ref.vertline.NP068832.1.vertline. **thymosin, beta,** 45 37/45 38/45 5e-06 identified in (82%) (84%) neuroblastoma cells [Homo sapiens] pir .vertline..vertline.I52084 **thymosin beta**-4 56 27/39 33/39 2e-04 precursor - rat (69%) (84%) (fragment) sp.vertline.P20065.vertline.TYB4_MOUSE **THYMOSIN BETA**-4 50 27/39 33/39 3e-04 (69%) (84%) gb.vertline.AAA36746.1.vertline. **thymosin beta**-10 49 24/40 32/40 0.002 (M92383) [Homo sapiens] (60%) (80%) gb.vertline.AAB37101.1.vertline. **thymosin beta**- 45 31/39 34/39 0.002 (U25684) like protein (79%) (86%) [Rattus norvegicus]

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TITLE: Human **thymosin beta15 gene,** protein and uses thereof

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INVENTOR-INFORMATION:

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APPL-NO: 09/ 874736

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child 09369744 19990806 US

parent division-of 09069484 19980429 US GRANTED

parent-patent 6017717 US

child 09069484 19980429 US

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child 08931877 19970917 US

parent division-of 08801796 19970214 US GRANTED

parent-patent 5721337 US

child 08801796 19970214 US

parent division-of 08664856 19960617 US GRANTED

parent-patent 5663071 US

US-CL-CURRENT: 530/388.26, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

---------- KWIC --------

Abstract Paragraph - ABTX (1):

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

Title - TTL (1):

Human **thymosin beta15 gene,** protein and uses thereof

Summary of Invention Paragraph - BSTX (9):

[0008] We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Summary of Invention Paragraph - BSTX (10):

[0009] The present invention provides isolated nucleic acids (polynucleotides) which encode **thymosin .b ta.15 having the deduced amino acid**

sequence of SEQ ID. NO: 2 or a unique fragment thereof. The term "unique fragment" refers to a portion of the nucleotide sequence or polypeptide of the invention that will contain sequences (either nucleotides or amino acid residues) present in thymosin .beta.15 (SEQ ID NO: 2) but not in other member of the thymosin family. This can be determined when the hybridization profile of that fragment under stringent conditions is such that it does not hybridize to other members of the thymosin family. Such fragments can be ascertained from FIG. 3. A preferred set of unique fragments are those that contain, or contain polynucleotides that encode, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2. Preferably, the unique nucleotide sequence fragment is 10 to 60 nucleotides in length, more preferably, 20 to 50 nucleotides, most preferably, 30 to 50 uncleotides. Preferably, the unique polypeptide sequence fragment is 4 to 20 amino acids in length, more preferably, 6 to 15 amino acids, most preferably, 6 to 10 amino acids.

Summary of Invention Paragraph - BSTX (14):

[0013] As used herein a polynucleotide "substantially identical" to SEQ ID NO:1 is one comprising at least 90% homology, preferably at least 95% homology, most preferably 99% homology to SEQ ID NO: 1. The reason for this is that such a **sequence can encode thymosin** .beta.15 in multiple mammalian species.

Summary of Invention Paragraph - BSTX (15):

[0014] The present invention further provides an isolated and purified human **thymosin .beta.15 having the amino acid sequence** of SEQ ID NO: 2, or a unique fragment thereof, as well as polypeptides comprising such unique fragments, including, for example, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2.

Summary of Invention Paragraph - BSTX (19):

[0018] The present invention further provides a method of treating a neoplastic cell expressing human thymosin .beta.15 by administering to the cell an effective amount of a compound which suppresses the activity or production of the human thymosin .beta.15. Preferably, the compound interferes with the expression of the human **thymosin .beta.15 gene**. Such compounds include, for example, antisense oligonucleotides, ribozymes, antibodies, including single chain antibodies and fragments thereof.

Brief Description of Drawings Paragraph - DRTX (2):

[0019] FIGS. 1A and 1B show differential mRNA display and Northern analysis of Dunning R-3327 rat prostatic adenocarcinoma variants. Total RNA from AT2.1 (lane 1), AT3.1 (lane 2) and AT6.1 (lane 3) cells were reverse-transcribed and amplified by PCR with a primer set, T.sub.11 AG and 10 mer AGGGAACGAG (SEQ ID NO:3) in the presence of [.alpha.35-S)dATP. The PCR fragments were displayed on a 6% polyarylamide gel and autoradiographed. The differentially expressed band is indicated by arrowhead. B. Northern blot analysis of **thymosin .beta.15 gene**. Two .mu.g of poly (A) RNA was isolated from Dunning R-3327 variants AT2.1 (lane 1), AT3.1 (lane 2), AT6.1 (lane 3), and Mat Lylu (lane 4), fracionated on a 1.1% formaldehyde-agarose gel, transferred to Hybond-N+ nylon

membrane (Amersham) and hybridized with a random primed (Grillon C, et al., FEBS 1990, 274:30-34) .sup.32P-labeled T.beta.15 cDNA fragment. The same blot was hybrodized with a rat .beta.-actin probe to demonstrate that equivalent amounts of RNA were loaded in each lane.

Brief Description of Drawings Paragraph - DRTX (11):

[0028] FIGS. 7A, 7B and 7C show serum stimulated migration of control transfected and T.beta.15 transfected Dunning R-3327 variants and their growth rate. FIG. 7A. Vector control transfected (.largecircle., .gradient.) and T.beta.15 antisense (.circle-solid., .tangle-soliddn.) transfected AT3.1 cell clones. FIG. 7B. Vector control transfected (.largecircle., .gradient.) and t.beta.15 sense transfected (.circle-solid., .tangle-soliddn.) AT2.1 cell clones. Data are expressed as the mean .+-.SE (n=4). FIG. 7C. Growth curves of control transfected and T.beta.15 (sense or antisense) transfected Dunning R-3327 clones. Cells from vector control transfected AT2.1 (.smallcircle.), T.beta.15 sense transfected AT2.1 (.circle-solid.), vector control transfected AT3.1 (.gradient.) and T.beta.15 antisense transfected AT3.1(.tangle-soliddn.) were plated at initial 10.sup.4 cells/well in RPMI 1640 with 10% FBS and 250 nM dexamethasome in 12-well plates. Cells were harvested and counted at indicated times. Points represent the mean .+-.SE (n=3). FIGS. 8A and 8B show Western analysis of **thymosin .beta.**-GST fusion protein. FIG. 8A is a Coomasie staining of GST-T.beta. fusion proteins. FIG. 8B is a Western analysis of GST-T.beta. fusion proteins with affinity purified anti-T.beta.15 C-terminal peptide antibody. Lane 1: GST-T.beta.4; Lane 2: GST-T.beta.15; Lane 3: GST only

Detail Description Paragraph - DETX (2):

[0032] A well characterized series of cell lines that show varying metastatic potential has been developed from the Dunning rat prostatic carcinoma (Isaacs, et al., Prostate 9, 261-281 and Bussebakers, et al., Cancer Res. 52, 2916-2922 (1992)). Coffey and colleagues previously showed a direct correlation between cell motility and metastatic potential in the Dunning cell lines (Mohler, et al., Cancer Res. 48, 4312-4317 (1988), Parin, et al., Proc. Natl. Acad. Sci, USA 86, 1254-1258 (1989) and Mohler, et al., Cancer Metast. Rev 12, 53-67 (1993). We compared gene expression in poorly metastatic and highly metastatic cell lines derived from Dunning rat prostate carcinoma using differential mRNA display. The results of these studies revealed the expression of a novel member of the **thymosin beta** family of actin-binding molecules, thymosin .beta.15. Using this information, we isolated and **sequenced a cDNA encoding human thymosin** .beta.15.

Detail Description Paragraph - DETX (3):

[0033] Although members of the **thymosin .beta.** family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that this new member, thymosin .beta.15, directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the

process of metastatic transformation.

Detail Description Paragraph - DETX (4):
[0034] The present invention provides a polynucleotide **sequence ncoding all or part of thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID NO:2 or a unique fragment thereof. A nucleotide **sequence encoding human thymosin** .beta.15 is set forth as SEQ ID NO:1.

Detail Description Paragraph - DETX (5):
[0035] The sequences of the invention may also be engineered to provide restriction sites, if desired. This can be done so as not to interfere with the peptide **sequence of the encoded thymosin** .beta.15, or may interfere to any extent desired or necessary, provided that the final product has the properties desired.

Detail Description Paragraph - DETX (27):
[0057] The antibody can be administered by a number of methods. One preferred method is set forth by Marasco and Haseltine in PCT WO94/02610, which is incorporated herein by reference. This method discloses the intracellular delivery of a **gene encoding the antibody, in this case the thymosin** .beta.15 antibody. One would preferably use a **gene encoding a single chain thymosin** .beta.15 antibody. The antibody would preferably contain a nuclear localization sequence, for example Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:4) [Lawrod, et al. Cell 46:575 (1986)]; Pro-Glu-Lys-Lys-Ile-Lys-Ser (SEQ ID NO:5) [Stanton, et al., Proc. Natl. Acad. Sci. USA 83:1772 (1986)], Gin-Pro-Lys-Lys-Pro (SEQ ID NO:6) [Harlow, et al., Mol. Cell. Biol. 5:1605 (1985)]; Arg-Lys-Lys-Arg (SEQ ID NO:7) for the nucleus. One preferably uses an SV40 nuclear localization signal. By this method one can intracellularly express a thymosin .beta.15 antibody, which can block thymosin .beta.15 functioning in desired cells.

Detail Description Paragraph - DETX (30):
[0060] Affecting **thymosin .beta.15 gene** expression may also be achieved more directly, such as by blocking of a site, ,such has the promoter, on the genomic DNA.

Detail Description Paragraph - DETX (35):
[0065] In addition, ribozymes can be used to inhibit in vitro expression of thymosin .beta.15. For example, the nucleic acids of the invention can further be used to design ribozymes which are capable of cleaving a single-stranded nucleic acid encoding a .beta.15 rpotein, such as a thymosin .beta.15 mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding **thymosin .beta.15 based upon the sequence** of a nucleic acid of the invention (e.g., SEQ ID NO: 1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base **sequence t be cleaved in a thymosin** .beta.15-encoding mRNA. See for example Cech, et al.,

U.S. Pat. No. 4,987,071; Cech, et al., U.S. Pat. No. 5,116,742. Alternatively, a nucleic acid of the invention could be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J. W. Science 261, 1411-1418 (1993).

Detail Description Paragraph - DETX (72):

[0099] To obtain a full-length complementary DNA (cDNA) clone of this gene, an AT3.1 cDNA library was screened using the originally cloned cDNA fragment from differential display as a probe. A positive clone with a 412 base pair insert was isolated, which contained a single open-reading frame encoding a 45 amino acid protein with a calculated molecular mass of 5304 (FIG. 2). The insert size of the clone was approximately the same as the molecular size of the transcript seen in Northern analysis suggesting that the clone contained the full length gene sequence. A computer assisted homology search against the Genebank and EMBL DNA databases revealed that the novel gene shared 49% nucleotide sequence homology with rat thymosins .beta.4 and .beta.10. Alignment of the deduced amino acid **sequence of the cloned gene with members of the thymosin .beta**. family (Mihelic, M. & Voelter, Amino Acids 6, 1-13 (1994) showed 68% homology with thymosin .beta.4, 62% with thymosin .beta.10 and 60% with .beta.9, .beta.11 and .beta.12 (FIG. 3). The results suggest that we have cloned a novel .beta.thymosin, now named thymosin .beta.15, from rat prostatic carciona cells.

Detail Description Paragraph - DETX (73):

[0100] Hydropathy analysis of the **thymosin .beta.15 protein sequence** revealed no apparent membrane-spanning or membrane-associated regions and no amino-terminal signal sequence. The protein is highly hydrophilic with an estimated isolectric point of 5.14 and contains regions common to all members of the **thymosin .beta**. family. All .beta.-thymosin family members previously studied, for example, have a putative actin binding region (LKKTET) 16 residues from the amino terminus (Vancompernolle, et al., EMBO J. 11, 4739-4746 (1992), Troys, et al., EMBO J. 15, 201-210 (1996). Thymosin .beta.15 also has such a region, although the glutamic acid residue is replaced by an asparagine residue to form LKKTNT (FIG. 3). The principal region of nonconformity between members of the **thymosin .beta**. family occurs at the carboxyl terminus and the **thymosin .beta.15 sequence** as well shows no significant homology in this region with other family members. j

Detail Description Paragraph - DETX (74):

[0101] Members of the .beta.-thymosin family may be independently expressed in different tissues (lin, et al., J. Biol. Chem. 266, 23347-23353 (1991), Voisin, et al. J. Neurochem. 64, 109-120 (1995). Although thymosin .beta.15 is differentially expressed in the prostate carcinoma cell lines tested, all of these lines expressed equivalent levels of thymosins .beta.4 and .beta.10 by RT-PCR analysis (FIG. 11). The tissue distribution of thymosin .beta.15 mRNA was examined in the major organs of the rat. No expression of thymosin .beta.15 was detected in the heart, brain, lung, spleen, liver, skeletal muscle and kidney, whereas high expression was found in the testis (FIG. 4). Southern (DNA) analysis of Hind III-, EcoRI- and Pst I-restricted DNA from AT2.1 and

AT3.1 cells with thymosin .beta.15 cDNA probe revealed no gross structural alteration of the **thym sin .beta.15 gene** in the tumor cells (data not shown). These results demonstrate that a novel member of the **thymosin .beta**. family is upregulated in metastatic rat prostatic carcinoma cell lines, whereas expression of other **thymosin .beta**. family members (.beta.4 and .beta.10) remains unchanged.

Detail Description Paragraph - DETX (77):

[0104] (5'-TATCAGCTAGTGGCTGCACCCGCG-3') (SEQ ID NO:8) and RI (5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR pofile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT-PCR were conducted using aliquats from the same samples and amplified with primers to the .beta.-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers was surprisingly 100% identical to the **thymosin .beta.15 sequence** obtained from the rat prostatic carcinoma cells.

Detail Description Paragraph - DETX (79):

[0106] To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified PCR product was 100% identical to the rat **thymosin .beta.15 sequence**. We conducted in situ hybridization study on samples from patients with varying grades of prostatic carcinomas using a thymosin .beta.15 probe. The tissue sections allowed direct comparison of normal and malignant elements on the same samples. The stromal elements within and around the tumor cell masses, as well as the nonmalignant prostatic epithelium adjacent to the tumor showed little background hybridization with the thymosin .beta.15 antisense probe. In contrast, specific tumor cell islands exhibited a strong specific thymosin .beta.15 signal when probed with antisense (FIG. 5A, small arrow) but not with a sense RNA probe (data not shown). Although nearly all of the tumor cells in the positive islands expressed thymosin .beta.15 mRNA, not all patient specimens were positive and not all islands in a single prostate were positive (FIG. 5A, large arrow). The majority of the negative tumor cells were in non-invasive in situ carcinomas whereas highly invasive tumors were consistently positive (FIG. 5B). Thus a novel .beta. thymosin, first detected in metastatic rat prostate cardinoma cell lines, is upregulated in human prostate cancer.

Detail Description Paragraph - DETX (83):

[0110] To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic

expression vector (pcDNA3) containing the **thym sin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thym sin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 7A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 7B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 7C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detail Description Paragraph - DETX (85):

[0112] A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone (FIG. 8) showing its specificity.

Detail Description Paragraph - DETX (89):

[0115] Progression to the metastatic stage is directly correlated with mortality from prostatic carcinoma. It therefore follows that the early diagnosis, prevention, or therapeutic treatment of metastatic progression would lead to more effective control of this disease. The Dunning R-3327 rat prostatic adenocarcinoma model provides several sublines with varying metastatic ability, all of which derive from an original spontaneous tumor and which provide an opportunity to study the steps leading to prostate cancer metastases (Mohler, Cancer Metast. Rev. 12, 53-67 1993) and Pienta, et al. Cancer Surveys 11, 255-263 (1993)). By comparing gene expression among the Dunning cells, we cloned a novel member of the **thymosin .beta**. family, thymosin .beta.15, which is expressed in highly metastatic prostate cancer cells but not in non- or weakly metastatic cells. The related family members thymosin .beta.4 and .beta.10 are expressed equally in all of the cell lines tested such that their expression does not vary with increasing metastatic potential.

Claims Text - CLTX (7):
6. An isolated and purified human **thymosin .beta.15 having the amino acid sequence** set forth in SEQ ID NO.: 2.

Claims Text - CLTX (9):
8. An isolated polynucleotide encoding human **thymosin .beta.15 comprising the amino acid sequence** as set forth in SEQ ID NO:2.

Claims Text - CLTX (15):
14. An isolated polynucleotide encoding human **thymosin .beta.15 having the nucleotide sequence** of nucleotides 98-232 of SEQ ID NO:1, or the complement thereto.

Claims Text - CLTX (19):
18. The method of claim 17, wherein the compound interferes with the expression of the human **thymosin .beta.15 gene**.

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US-CL-CURRENT: 514/44, 435/287.2 , 435/6

ABSTRACT:

Implementing gene expression to study drug action in the central nervous system is complicated by functional heterogeneity because of the existence of many different neuronal subtypes within the mammalian brain. The integration of laser capture microdissection (LCM) and RNA amplification with cDNA microarray technology allows for large-scale gene expression analysis at cellular level. Using this approach, we have generated gene expression profiles of imipramine, a reference antidepressant, and a new putative antidepressant, novelR1 in several laser-captured brain nuclei (locus coeruleus, dorsal raphe, hypothalamic paraventricular nucleus and hippocampus) of rats subjected to the chronic mild stress model (CMS) of depression.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Provisional Application Ser. No. 60/238,374, filed on Oct. 6, 2000, and U.S. Provisional Application Ser. No. 60/295,782, filed on Jun. 4, 2001.

---------- KWIC ---------

Detail Description Table CWU - DETL (7):
100763.vertline. 100763.vertline. P11661 Rat nadh-ubiquinone ox 182
neuropeptide 38737.vertline. 701905916.vertline. M10088.vertline. dynorphin
183 G protein signaling 38729.vertline. 95348.vertline. 95348.vertline.
GTP-binding protein rab10 - rat 184 38725.vertline. 102725.vertline.
102725.vertline. Novel 185 ion channel 38721.vertline. 700247271.vertline.
L08495.vertline. GABA receptor alpha6 186 GPCR 38716.vertline.
701031976.vertline. X63574.vertline. Somatostatin 3 Rec 187 38714.vertline.
112210.vertline. 112210.vertline. Mouse LB9 gene, partial sequence. 188
38712.vertline. 669616.vertline. U89412.vertline. PML isoform 1 189
38711.vertline. 716499.vertline. U90724.vertline.
X-Proaminopeptidase(eukaryote) 190 GPCR 38710.vertline. 700369402.vertline.
L14618.vertline. Calcitonin Rec 191 38701.vertline. 1331673.vertline.
M11597.vertline. CGRP alpha 192 38694.vertline. 112194.vertline.
112194.vertline. Novel 193 38676.vertline. 701896910.vertline.
M36418.vertline. GLR1 194 GPCR 38675.vertline. 701901569.vertline.
M99376.vertline. Adrenergic alpha2C Rec 195 38667.vertline. 111664.vertline.
111664.vertline. Human (clone hh18) protein tyrosine phospha 196
38664.vertline. 700133895.vertline. 038450.vertline. 954 Rec 197
38663.vertline. 701921173.vertline. M64752.vertline. glutamate rec sub (GluH1)
198 38653.vertline. 106117.vertline. 106117.vertline. Novel 199
38623.vertline. 113304.vertline. 113304.vertline. Rat light molecular-weight
neurofilament 200 38621.vertline. 444406.vertline. 444406.vertline. Novel
201 38614.vertline. 111621.vertline. 111621.vertline. Novel 202
38605.vertline. 108620.vertline. 108620.vertline. Rat receptor for
hyaluronan-mediated 203 38602.vertline. 101693.vertline. 101693.vertline.
Mouse ribosomal protein (ke-3) gene, exons 204 38601.vertline.
100748.vertline. 100748.vertline. Mouse transciptional regulator RPD3 homolo
205 38594.vertline. 113748.vertline. 113748.vertline. Mouse necdin mRNA,
complete cds. 206 ion channel 38582.vertline. 700773668.vertline.
M81253.vertline. Kv3.4 gene 207 38577.vertline. 113515.vertline.
113515.vertline. Novel 208 38558.vertline. 66940.vertline. 66840.vertline.
Novel 209 38546.vertline. 96780.vertline. 96780.vertline. Bos taurus clone
CSSM015 microsatellite DNA sequen 210 38545.vertline. 101138.vertline.
101138.vertline. Rat ribosomal protein S24 211 38536.vertline.
111254.vertline. 111254.vertline. Human C14orf3 protein 212 38533.vertline.
475889.vertline. AF020198.vertline. PBX2 213 38532.vertline. 103937.vertline.
103937.vertline. Rat heavy neurofilament (NF-H) polypeptide, partia 214
38528.vertline. 112216.vertline. 112216.vertline. S-100 beta subunit [rats,
Genomic, 423 nt, segment 215 38522.vertline. 463924.vertline. T67463.vertline.
other peptidase (C1A) 216 38508.vertline. 66531.vertline. 66531.vertline.
Novel 217 38504.vertline. 84486.vertline. 84486.vertline. Novel 218
38491.vertline. 108519.vertline. 108519.vertline. Rat gamma-smooth muscle
isoactin pro 219 38487.vertline. 112021.vertline. 112021.vertline. Rat brain
ID transcript, clone bBC2- 220 38469.vertline. 95406.vertline.
95406.vertline. Rat light molecular-weight neurofilament 221 38463.vertline.
113514.vertline. 113514.vertline. Novel 222 38417.vertline. 79183.vertline.
79183.vertline. Novel 223 38404.vertline. 1039810.vertline.

AA297071.vertline. Similar to N-methyl-D-aspartate glutamate receptor 224
38402.vertline. 701289315.vertline. X99792.vertline. capacitative Ca entry
channel 1 225 ion channel 38386.vertline. 700495245.vertline.
L08493.vertline. GABA-A receptor 226 37466.vertline. 386514.vertline.
W65013.vertline. Similar to X07384 GLI PROTEIN. 227 37464.vertline.
386559.vertline. W65005.vertline. Mouse cDNA clone IMAGE:386559 5' 228
36393.vertline. 387565.vertline. A18932401 Similar to L14935 Mouse neural
retina-specific leucine zipper protein. 229 ion channel 33.vertline.
426170.vertline. AA002993.vertline. Potassium channel Kv3.4a (Raw3), V-gated
230 2319.vertline. UI-R-A0-af-e-06-0-UI.vertline. AA817907.vertline. Similar
to MUSCDPK mouse cyclin-dependent kinase homologue (p130PITSL) mRNA, complete
cdsmRNA 231 21709.vertline. UI-R-AF0-ya-b-04-0-UI.vertline.
988064T6.vertline. LVENNOT03 INCYTE 232 21692.vertline.
UI-R-AC1-xn-e-07-0-UI.vertline. 817852H1.vertline. OVARTUT01 INCYTE 233
21664.vertline. 903057.vertline. 580795H1.vertline. NMDA channel-like 234 DNA
repair 21573.vertline. 1054114.vertline. 3764982H1.vertline. human homologue
of yeast RAD23 235 DNA repair 21458.vertline. UI-R-E0-ci-a-06-0-UI.ve-
rtline. 2583919H1.vertline. human homologue of yeast RAD23 236
21418.vertline. 1348327.vertline. 2049923H1.vertline. LIVRFET02 U43892 g1
167981 Mouse ABC transporter-7 mRNA, partial cd gb103rod 83 -55 237 DNA repair
21304.vertline. 420048.vertline. 1676240T6.vertline. cell cycle checkpoint
rad9 gene 238 21251.vertline. 975800.vertline. 1275842F6.vertline. Eph-Erk
239 ion channel 21230.vertline. 748734.vertline. 2658404T6.vertline. SK2
calcium-activ potassium chann homolo 240 20977.vertline.
UI-R-02p-np-g-01-0-UI.vertline. 485639T6.vertline. retinoic acid hydroxylase
hP450RAI 241 20971.vertline. 2182407.vertline. 3369279H1.vertline. LON;
ATP-Dep Proteas LA 242 20768.vertline. 96782.vertline. 96782.vertline.
M58405 R. norvegicus **thymosin beta-10 gene,** complete cds. 1.0e-72 243
20765.vertline. 95816.vertline. 95816 X51396 Mouse MAP1B mRNA for MAP1B
microtubule-associated 6.5e-99 244 axon outgrowth 20763.vertline.
95791.vertline. 95791.vertline. M16228 Rat neuronal growth protein 43 (GAP-43)
mRNA, comp 9.7e-187 245 20762.vertline. 95494.vertline. 95494.vertline.
M93056 Human mononcyte/neutrophil elastase inhibitor mRNA 1.9e-117 246
20760.vertline. 95467.vertline. 95467.vertline. U43747 Human frataxin (FRDA)
mRNA, complete cds. 1.9e-107 247 20759.vertline. 95416.vertline.
95416.vertline. S77858 non-muscle myosin alkali light chain [rats, Spragu
2.9e-92 248 hsp 20754.vertline. 95386.vertline. 95386.vertline. M11942 Rat 70
kd heat-shock-like protein mRNA, complete c 9.0e-108 249 20751.vertline.
95321.vertline. 95321.vertline. G27093 human STS SHGC-31976. 1.5e-91 250
20749.vertline. 95308.vertline. 95308.vertline. S82024 SCG10=neuron-specific
growth-associated protein/st 4.4e-102 251 20748.vertline. 94433.vertline.
94433.vertline. Z92839 Caenorhabditis elegans cosmid T08D2, complete sequ 0.89
252 20743.vertline. 84483.vertline. 84483.vertline. AC003043 Homo sapiens
chromosome 17, clone HRPC1067M6, comp 2.9e-31 253 energy production
20740.vertline. 83925.vertline. 83925.vertline. X02231 Rat mRNA for
glyceraldehyde-3-phosphate-dehydrogen 7.9e-210 254 20739.vertline.
83922.vertline. 83922.vertline. 569874 C-FABP=cutaneous fatty acid-binding
protein [rats, 6.0e-194 255 20738.vertline. 83912183912.vertline. L37086 Homo
sapiens FK-506 binding protein (fkbp12.6) gen 1.9e-151 256 20726.vertline.
82552.vertline. 82552.vertline. D17296 Rat mRNA for polyubiquitin (ten
completely repetit 5.4e-90 257 20724.vertline. 82519.vertline.
82519.vertline. M81225 Rat farnesyltransferase alpha subunit mRNA,
comple2.7e-164 258 20720.vertline. 82475.vertline. 82475.vertline. V00681 R.

norvegicus mitochondrial genes for 16S rRNA, tRN 8.6e-82 259 20716.vertline. 82210.vertline. 82210.vertline. AF014955 Homo sapiens TFAR19 mRNA, complete cds. 1.3e-70 260 20710.vertline. 82096.vertline. 82096.vertline. X14848 Rat (R. norvegicus) mitochondrial genome. 1.0e-99 261 2071.vertline. UI-R-AO-at-f-11-0-UI.vertline. AA818381.vertline. Similar to MMCKIT Mouse c-kit mRNAmRNA 262 axon outgroth 20709.vertline. 82094.vertline. 82094.vertline. X03369 Rat mRNA for beta-tubulin T beta15. 5.7e-105 263 20708.vertline. 82093.vertline. 82093.vertline. G15458 human STS SHGC-16876. 6.7e-51 264 20703.vertline. 82047.vertline. 82047.vertline. M71245 Rat prostatein C3 subunit gene, complete cds. 3.7e-36 265 proteasome 20694.vertline. 81776.vertline. 817761045249 Rat mRNA for proteasome activator rPA28 subunit al 1.4e-178 266 20693.vertline. 81773.vertline. 81773.vertline. X68273 M. musculus mRNA for macrosialin. 1.0e-134 267 20690.vertline. 81743.vertline. 81743.vertline. X05608 Human gene for neurofilament subunit NF-L. 5.0e-38 268 20674.vertline. 80860.vertline. 80860.vertline. X95591 M. musculus mRNA for C1D protein. 1.7e-77 269 20673.vertline. 80835.vertline. 80835.vertline. AC000083 Home sapiens Chromosome 22q11.2 Cosmid Clone 68a1 0.65 270 20672.vertline. 80832.vertline. 80832.vertline. U16686 Rattus norvegicus defensin RatNP-1 precursor mRNA, 2.9e-117 271 20669.vertline. 80637.vertline. 80637.vertline. X70369 R. norvegicus mRNA for pro alpha 1 collagen type II 2.9e-81 272 20667.vertline. 80178.vertline. 80178.vertline. M58405 R. norvegicus **thymosin beta**-b gene, complete cds. 6.1e-79 273 20665.vertline. 80157.vertline. 80157.vertline. D83407 ZAKI-4 mRNA in human skin fibroblast, complete cds 1.2e-59 274 20661.vertline. 80013.vertline. 80013.vertline. G27219 human STS SHGC-30611. 2.6e-60 275 20655.vertline. 79192.vertline. 79192.vertline. V00680 Rat mitochondrial genes coding for 16S and 12S rRN 1.7e-109 276 20654.vertline. 79184.vertline. 79184.vertline. J04517 Rat high molecular weight neurofilament (NF-H) pro 5.3e-141 277 20591.vertline. 66897.vertline. 66897.vertline. M80899 Human novel protein AHNAK mRNA, partial sequence. 1.3e-38 278 20590.vertline. 66886.vertline. 66886.vertline. U52828 Human Cri-du-chat region mRNA, clone NIBA2. 2.2e-75 279 20588.vertline. 66841.vertline. 66841.vertline. X51406 Rat mRNA for carboxypeptidase E (EC 3.4.17.10). 2.4e-167 280 20587.vertline. 66839.vertline. 66839.vertline. M15563 Human MHC class II HLA-DR-beta-1 pseudogene (DR7), 0.79 281 20583.vertline. 66706.vertline. 66706.vertline. M30952 Orangutan 28S ribosomal RNA gene fragment. 9.8e-151 282 20582.vertline. 66692.vertline. 66692.vertline. L81910 Homo sapiens (subclone 3_c10 from PAC H74) DNA seq 0.00048 283 20581.vertline. 66688.vertline. 66688.vertline. U49099 Rattus nervegicus cis-Golgi p28 (p28) mRNA, comle 6.8e-193 284 20568.vertline. 115932.vertline. 115932.vertline. M25638 Rat smallest neurofilament protein (NF-L) mRNA, pa 6.7e-157 285 20566.vertline. 115916.vertline. 115916.vertline. X62952 R. norvegicus mRNA for vimentin. 2.8e-167 286 20563.vertline. 113752.vertline. 113752.vertline. AL021308 Human DNA sequence from cosmid U246D9 on chromosom 0.93 287 20562.vertline. 113749.vertline. 113749.vertline. M80840 M. musculus necdin mRNA, complete cds. 1.7e-71 288 20557.vertline. 113571.vertline.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010044414 A1

TITLE: Metastasis genes and uses thereof

PUBLICATION-DATE: November 22, 2001

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APPL-NO: 09/ 735273

DATE FILED: December 11, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60170233 19991210 US

US-CL-CURRENT: 514/44, 435/6 , 435/7.23

ABSTRACT:

The identification of a subset of genes which function in metastasis of tumor cells is described. Also described are methods of diagnosis and therapy of metastatic conditions relating to the identified genes.

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 60/170,233 filed Dec. 10, 1999, the entire teachings of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):

[0003] Metastasis, the process whereby tumor cells migrate throughout the body, is complex. In order for a tumor to produce metastases it must contain cells of the correct genotype be capable of completing a complex series of steps. The steps of tumor cell metastasis include the detachment of tumor cells from the primary neoplasm, invasion into the surrounding stroma, intravasation into the vasculature or lymphatic system, survival in the circulation, extravasation into the new host organ or tissue, and then survival

and growth in this new microenvironment (Van Noorden et al., 1988). Specific genes are likely to control specific events at each of these steps; however, to date, relatively few genes have been implicated in the process of tumor metastasis. Nm23, KiSS1, CD82/KAI1, E-cadherin, and thrombospondin 1 have been identified as genes capable of suppressing metastasis in various experimental tumor models (Fidler and Radinsky, 1966; Roberts, 1996), while ras, CD44, **thymosin .beta.15, and Tiam1 are among the genes** capable of inducing metastasis (Vousden et al., 1986; Sherman et al., 1994; Bao et al., 1996; Habets et al., 1994). While these studies have enhanced the understanding of metastasis, they provide only a partial picture of such a complex system.

Summary of Invention Paragraph - BSTX (6):

[0006] Thus, the invention relates to a method of inhibiting metastasis in a mammal, e.g., a human, comprising administering to a mammal in need thereof an effective amount of an agent which alters the actin-based cytoskeleton of one or more cells in the mammal. In one embodiment, the agent inhibits formation of the actin-based cytoskeleton. In a particular embodiment, the agent inhibits the activity of a gene selected from the group consisting of the **genes encoding fibronectin, RhoC, thymosin** .beta.4, t-PA, angiopoietin 1, IEX-1/Glu96, RTP/NDR1, fibromodulin, Hsp70, IL13 Rec. .alpha.2, Sec61.beta., snRNP polypeptide C, collagen I.alpha.2, UBE21, KIAA0156, TGF.beta. superfamily, surfactant protein C, lysozyme M, matrix Gla protein, Tsa-1, collagen III.alpha.1, biglycan, .alpha.-catenin, valosin-cont. prot., ERK-1, .alpha.-actinin 1, calmodulin, EIF4.gamma., .alpha.-centractin, IQGAP1, cathepsin S, EF2, and the genes in Table 5. In another particular embodiment, the agent inhibits the gene encoding RhoC. The agent can inhibit the activity of the gene directly or by inhibiting the activity of a downstream effector of the gene. For example, the agent can be a nucleic acid molecule (e.g., one or more antisense molecules or nucleic acid molecules encoding one or more dominant negative form of a gene product), an antibody, a peptide, an organic molecule, an inorganic molecule, or any combination of two or more of the preceding (e.g., two or more nucleic acid molecules; a nucleic acid molecule(s) and an organic molecules(s)).

Detail Description Paragraph - DETX (10):

[0027] In another embodiment, the present invention provides a method of inhibiting metastasis in a mammal comprising the inhibiting the activity of one or more genes selected from the group consisting of the **genes encoding fibronectin, RhoC, thymosin** .beta.4, t-PA, angiopoietin 1, IEX-1/Glu96, RTP/NDR1, fibromodulin, Hsp70, IL13 Rec. .alpha.2, Sec61.beta., snRNP polypeptide C, collagen I.alpha.2, UBE21, KIAA0156, TGF.beta. superfamily, surfactant protein C, lysozyme M, matrix Gla protein, Tsa-1, collagen III.alpha.1, biglycan, a-catenin, valosin-cont. prot., ERK-1, .alpha.-actinin 1, calmodulin, EIF4.gamma., .alpha.-centractin, IQGAP1, cathepsin S, EF2, and the genes listed in Table 5. The agent may inhibit transcription of the gene, alter (render non-translatable) or degrade the transcript, or inhibit the activity of the encoded gene product.

Detail Description Paragraph - DETX (48):

[0064] The human fibronectin (Genbank accession number X02761), rhoC

(L25081), and **thym sin .beta.4 (M17733) genes** were cloned using a Zero Blunt TOPO PCR Cloning Kit (In Vitrogen) according to the manufacturers instructions. PCR fragments for cloning were generated with vent polymerase as follows: for fibronectin, a 425 base pair (bp) fragment (nucleotides 6848 to 7273) was synthesized using the primers GTCCCGAAGGCACTACT (SEQ ID NO: 1) and ATCCCAAACCAAATCTTA (SEQ ID NO: 2), for rhoC a 626 bp fragment (nucleotides -3 to 623) was synthesized using the primers ACCATGGCTGCAATCCGAAAGAAG (SEQ ID NO: 3) and AAGGGAGGGGGCATGTAGGAAAAG (SEQ ID NO: 4); and for thymosin .beta.4 a 405 bp fragment (nucleotides -28 to 377) was synthesized using the primers CGCCTCGCTTCGCTTTTC (SEQ ID NO: 5) and CACCCCACTTCTTCCTTCACCA (SEQ ID NO: 6).
For rhoC and thymosin .beta.4, the PCR fragments contain the entire coding region and significant 3' sequence. After cloning into the pCR-BluntII TOPO vector, the PCR products were sequenced to confirm the sequence obtained.

Detail Description Paragraph - DETX (65):
[0081] The data shown in the top half of Table 1 is the subset of genes expressed at consistently higher levels in the pulmonary metastases (M1, M2, and SM) when compared to the poorly-metastatic A375P tumor. Genes expressed at higher levels in the pulmonary metastases generated from the mouse B16 line (F1, F2, and F3) when compared to the poorly-metastatic B16F0 tumor are shown in the lower half of Table 1. Three **genes, fibronectin, rhoC, and thymosin** .beta.4, were expressed at higher levels in all three metastases selected from both the human A375 and mouse B16 cell lines, suggesting that their altered expression may be important for tumor metastasis. Enhanced expression of these three genes in the pulmonary metastases was confirmed by RNAse protection (FIG. 2).

Detail Description Paragraph - DETX (89):
[0105] Hall, A. K. Differential expression of **thymosin genes** in human tumors and in the developing human kidney. Int. J. Cancer 48 672 (1991).

Detail Description Paragraph - DETX (110):
[0126] Weterman, M. A. J., van Muigen, G. N. P., Ruter, D. J., & Bloemers, H. P. J. **Thymosin .beta.**-10 expression in melanoma cell lines and melanocytic lesions; a new progression marker for human cutaneous melanoma. Int. J. Cancer 53 278-284 (1993).

Detail Description Table CWU - DETL (5):
5TABLE 5 ADDITIONAL HETASTASIS GENES Additional Genes Cytochrome c-1 Peptidylprolyl isomerase B (cyclophilin B) CD58 antigen (lymphocyte function-associated antigen 3) Splicing factor, SF1-Bo isoform Putative serine/threonine protein kinaae (Y10032) CID protein Annexin .perp. (lipocortin) TIMP-3 Tubby homolog Protein tyrosine phosphatase, non-receptor type 12 NAD-dependent methylene tetrahydrofolate dehydrogenase cyclohydrolase Tissue inhibitor of metalloproteinase 2 Syntaxin-16C Clone RES4-24A Protein tyrosine phosphatase PTPCAAX1 Mucin GIF CDC28 protein kinase 2 Squalene epoxidase OZF CD9 antigen Arginine-rich protein (ARP) Calponin 3, acidic Metalloproteinase inhibitor 3 precursor Small nuclear

ribonucleoprotein polypeptide B Lysyl hydroxylase Induced myeloid leukemia cell differentiation protein MCL1 Chromosome 17q21 mRNA clone LF113 Putative transmembrane protein (nma) Laminin, gamma 1 Chromosome segregation gene homolog CAS KIAA0156 gene Chondroitin sulfate proteoglycan 2 (versican) RagA protein Lumican TAPA-1 KIAA0170 Cytoplasmic Dynein light chain 1 Insulin-like growth factor binding protein 3 precursor Factor VII serine protease precursor Laminin, alpha 4 Sp1 transcription factor Apolipoprotein D Elastin Annexin II RP3 MUC18 BAT3 SPARC/osteonectin Translation elongation factor 1 gamma Eukaryotic translation initiation factor 4A Superoxide dismutase 3 **Thymosin beta**-10 autotaxin Succinate dehydrogenase, iron sulfur subunit Heterogeneous nuclear ribonucleoprotein A2/B1

Claims Text - CLTX (5):

4. A method of inhibiting metastasis in a mammal, comprising administering to a mammal in need thereof an effective amount of an agent wherein said agent inhibits the activity of one or more genes selected from the group consisting of the **genes encoding fibronectin, RhoC, thymosin** .beta.4, t-PA, angiopoietin 1, IEX-1/Glu96, RTP/NDR1, fibromodulin, Hsp70, IL13 Rec. .alpha.2, Sec61.beta., snRNP polypeptide C, collagen I.alpha.2, UBE21, KIAA0156, TGF.beta. superfamily, surfactant protein C, lysozyme M, matrix Gla protein, Tsa- 1, collagen III.alpha.1, biglycan, .alpha.-catenin, valosin-containing protein, ERK-1, .alpha.-actinin 1, calmodulin, EIF4.gamma., .alpha.-centractin, IQGAP 1, cathepsin S and EF2 such that metastasis is inhibited.

US-PAT-NO: 6489463

DOCUMENT-IDENTIFIER: US 6489463 B1

TITLE: **Thymosin .beta.**-15 promoter and uses thereof

DATE-ISSUED: December 3, 2002

INVENTOR-INFORMATION:

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Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 09/ 549052

DATE FILED: April 13, 2000

PARENT-CASE:

This application is a continuation of International Application PCT/US98/21671 filed on Oct. 18, 1998 and which designated the United States, which claims the benefit of U.S. Provisional Application No. 60/062,969 filed Oct. 16, 1997 now abandoned.

US-CL-CURRENT: 536/24.1, 435/320.1

ABSTRACT:

The present invention provides a thymosin .beta.15 promoter. The promoter comprises the nucleotide sequence of SEQ ID NO:1 or a fragment thereof capable of expressing an operably linked DNA. A DNA sequence having nucleotides -400 to +1 of FIG. 1 is a preferred fragment. The invention also provides novel assays for identifying compounds useful in the treatment of malignancies involving modulation of thymosin .beta.15 expression, e.g., breast, pancreas and prostate cancer. The invention further provides a method of human gene therapy for treating malignancies involving up-regulation of thymosin .beta.15 expression.

7 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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TITLE - TI (1):
Thymosin .beta.-15 promoter and uses thereof

Brief Summary Text - BSTX (5):

Beta thymosins are a family of closely related, highly polar 5 kDa polypeptides. All vertebrates studied and some invertebrates are known to contain one or often two beta thymosins (Nachmias VT, Curr. Opin. Cell Biol., 1993;56-62). **Thymosin .beta.15 was recently uncovered in a search for genes** with increased expression in motile as compared to poorly motile Dunning rat prostatic carcinoma cell lines (Bao, et al., Nature Medicine, 1996; 1322-1328). The protein, which is 5300 Da, was designated thymosin .beta.15 because of its approximately 60% homology with other members of the beta thymosin family.

Brief Summary Text - BSTX (7):

The putative role of beta thymosin in modulation of the actin cytoskeleton through monomer sequestration suggests that they may be involved in cell differentiation, carcinogenesis and metastasis. In some cell lines, increased thymosin .beta.4 protein or mRNA has been shown to correlate with differentiation, while in most others it has not (Safer, et al., Bioessays, 1994; 16:473-9). In human tumors, thymosin .beta.4 mRNA has been shown to be increased in hairy cell leukemia and reduced in some lymphomas (Otero, et al., Biochem Biophys. Acta., 1993; 1176:59-63). Two out of three metastatic colorectal carcinomas showed decreased thymosin .beta.4 mRNA compared to non-metastatic tumors with the third metastatic tumor showing little change (Yamamoto, et al., Biochem Biophys. Res. Commun, 1993; 193:706-10). Thymosin .beta.10 mRNA levels are increased in renal cell carcinomas (Hall, AK, Ren. Fail, 1994; 16:243-54, Hall, AK, Cell Mo. Biol. Res. Commun, 1995;41:167-80), and thymosin .beta.10 up-regulation was shown to correlate with the metastatic potential of melanomas (Weterman, et al., Int. J Cancer, 1993; 53:278-84). The expression of each **thymosin beta** family member is independently regulated. Consequently different family members may be independently elevated or decreased in particular tumor types. As thymosin .beta.15 has only recently been described, it is less well characterized. It is present in very few normal adult tissues but was shown to be up-regulated in metastatic human prostate cancers at both the mRNA and protein level as compared to less metastatic prostate cancers (Bao, et al.). Immunostaining of human prostate cancer cases revealed a general correlation between Gleason grade and thymosin .beta.15 expression, with high grade tumors (Gleason grade 8-10) showing increased staining compared to low grade tumors (Gleason grade 2-5).

Brief Summary Text - BSTX (12):

The invention also provides novel assays for identifying compounds useful in the treatment of malignancies involving modulation of thymosin .beta.15 expression, e.g., breast, pancreas and prostate cancer. Preferred compounds identified through assays of the invention can modulate, particularly inhibit, thymosin .beta.15. A preferred assay comprises transfecting a host cell with a vector containing the **thymosin .beta.15 promoter operably linked to a gene** encoding a reporter or marker protein; contacting the cell with a test compound and measuring reporter protein expression.

Brief Summary Text - BSTX (13):

The invention also provides a method of human gene therapy for treating malignancies involving up-regulation of thymosin .beta.15 expression, e.g., breast, pancreas and prostate cancer. The method comprises administering to a human in need thereof an expression vector comprising the thymosin .beta.15 promoter operably linked to a DNA encoding a **gene product the expression of which by the cell expressing thymosin** .beta.15 , i.e., the cancer cells, inhibits the growth of the cell or results in the cells death. Such gene products include, for example, toxins, suicide genes, ribozymes, intrabodies, or antisense DNA or RNA.

Detailed Description Text - DETX (9):

For example, as indicated above, with PEA only Domain III is absolutely required. However, partial sequences from other domains makes the toxin more effective. For example, one can prepare PEA mammalian expression vectors in which Domain III (mature PEA amino acid residues 405 to 613) only, is expressed and one which encodes Domain III and partial Domain IB, a sequence of amino acids 385 to 613 is expressed. These **sequences should be operably linked to the thymosin** .beta.15 promoter, which will permit expression in the target cell. The toxin proteins encoded by these gene fragments lack a recognition domain. They are non-toxic to surrounding cells and are only toxic when expressed inside a cell. These expression vectors can readily be tested to determine how well they express a product intracellularly by a simple in vitro assay. For example, the expression of those DNA sequences encoding PEA toxin fragments can be tested by transforming .beta.15 expressing malignant cells with the vector and observing the cytotoxicity of the cell. Suicide genes such as tk work by sensitizing the cell to a compound which the cell would otherwise not be affected by, e.g. ganciclovir. One preferred type of antibodies works by binding to a target intracellularlly (e.g. an intrabody). For example, by targeting a **gene product that is overexpressed in malignant cells such as thymosin** .beta.15.

Detailed Description Text - DETX (16):

A preferred assay mixture of the invention comprises a host cell transfected with a vector containing the **thymosin .beta.15 promoter operably linked to a gene** encoding a reporter or marker protein. Preferred marker proteins include reporters such as .beta.-galactosidase, chloramphenicol acetyltransferase (CAT) and luciferase. An assay mixture of the invention also comprises a candidate pharmacological agent. Generally a plurality of assay mixtures are run in parallel with different candidate agent concentrations to obtain a differential response to the various concentrations. Typically, one of these assay mixtures serves as a negative control, i.e. at zero concentration or below the limits of assay detection. Candidate agents encompass numerous chemical classes, though typically they are organic compounds and preferably small organic compounds. Small organic compounds suitably may have e.g. a molecular weight of more than about 50 yet less than about 5,000. Candidate agents may comprise functional chemical groups that interact with proteins and/or DNA.

Detailed Description Text - DETX (32):

To obtain the promoter region of **thymosin .beta.15 gene,** a PCR-based GenomeWalker method was used. The GenomeWalker genomic libraries constructed from AT3. 1 cells were amplified using an adaptoe-specific sense primer (Olontech) and an antisense primer specific for exon 1 of thymosin .beta.15. Subsequent amplification of the PCR products with nested primers generated single major products, with an about 500-bp product in Dra I library and a 1.8-kb product in EcoRV library. The sequence of the product from Dra I library was identical to the sequence of 3'-region of the 1.8-kb product from EcoRV library, suggesting that they represent the same thymosin .beta.15 genomic DNA rather than distinct genes. The nucleotide sequence of the product from Dra I library is shown in FIG. 1 Examination of this sequence revealed that within the putative promoter region of 300 bp upstream of exon 1, the sequences were GC-rich (60%) and showed the presence of the following motifs: (i) a canonical SP1 binding site, (ii) a potential binding site for the DNA binding factor CREB and (iii) a putative CAAT box. No prototypical TATA box appeared to be present. This suggests that expression of the **thymosin .beta.15 gene** may be controlled by a typical housekeeping gene promoter.

Detailed Description Text - DETX (33):

To localize the transcription start site of **thymosin .beta.15 gene,** primer extension analysis was performed with an antisense oligonucleotide and the total RNA from AT3. 1 cells as a template. The major extension product was observed as a band about 60 bp in length.

Detailed Description Text - DETX (35):

To verify whether the genomic fragment flanking the 5' end of rat thymosin .beta.15 cDNA has promoter activity, a genomic fragment corresponding to bp -570 to 60 was coupled to a promoterless luciferase transcription reporter gene and examined for the ability to mediate basal transcription. The rat prostatic carcinoma cell line, AT3. 1, was transiently transfected with reporter constructs, and cell extracts were assayed for luciferase activity 48 h post-transfection. **Thymosin .beta.15 sequences** fused to the reporter in the appropriate transcriptional orientation (pGL2-5') consistently produced an about 100 fold increase in luciferase activity when compared to a promoterless luciferase construct (pGL2-basic). In contrast, **thymosin .beta.15 sequences** fused in the opposite transcriptional orientation (PGL2-5' reverse) did not induce a significant level of reporter activity. These results indicate that the 5'-flanking region has a promoter activity. In addition, we also examined the effect of TPA and cpt-cAMP on promoter activity of the 5'-flanking region. TPA (10.sup.-6 M) up-regulated luciferase activity about 2-fold in pGL2--5', whereas cpt-cAMP (200 mM) had no effect.

Other Reference Publication - OREF (1):

Bao, L, et al, 2000, Molecular cloning and structural characterization of the rat **thymosin beta15 gene,** Gene, vol. 260, No. 1-2, pp. 37-44*

Other Reference Publication - OREF (5):

S. Varghese et al., 1991, "Rat **Thymosin Beta 4 Gene,**" 266(22):14256-14261.

US-PAT-NO: 6300479

DOCUMENT-IDENTIFIER: US 6300479 B1

TITLE: Antibodies specific for human thymosin .beta.15 protein and uses thereof

DATE-ISSUED: October 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 09/ 369744

DATE FILED: August 6, 1999

PARENT-CASE:

This application is a divisional of application Ser. No. 09/069,484 filed on Apr. 29, 1998, now U.S. Pat. No. 6,017,717 which is a divisional of application Ser. No. 08/931,877 filed on Sep. 17, 1997 now U.S. Pat. No. 5,831,033, granted Nov. 3, 1998, which is a divisional of application Ser. No. 08/801,796 filed on Feb. 14, 1997, now U.S. Pat. No. 5,721,337, granted Feb. 24, 1998, which is a divisional of application Ser. No. 08/664,856 filed on Jun. 17, 1996, now U.S. Pat. No. 5,663,071, granted Sep. 2, 1997.

US-CL-CURRENT: 530/387.9, 530/387.1 , 530/387.3 , 530/388.1

ABSTRACT:

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

7 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Abstract Text - ABTX (1):

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

Government Interest Text - GOTX (1):

HUMAN **THYMOSIN .beta.15 GENE,** PROTEIN AND USES THEREOF

Brief Summary Text - BSTX (9):

We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (10):

The present invention provides isolated nucleic acids (polynucleotides) which encode **thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID. NO: 2 or a unique fragment thereof. The term "unique fragment" refers to a portion of the nucleotide sequence or polypeptide of the invention that will contain sequences (either nucleotides or amino acid residues) present in thymosin .beta.15 (SEC ID NO: 2) but not in other member of the thymosin family. This can be determined when the hybridization profile of that fragment under stringent conditions is such that it does not hybridize to other members of the thymosin family. Such fragments can be ascertained from FIG. 3. A preferred set of unique fragments are those that contain, or contain polynucleotides that encode, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2. Preferably, the unique nucleotide sequence fragment is 10 to 60 nucleotides in length, more preferably, 20 to 50 nucleotides, most preferably, 30 to 50 nuceotides.. Preferably, the unique polypeptide sequence fragment is 4 to 20 amino acids in length, more preferably, 6 to 15 amino acids, most preferably, 6 to 10 amino acids.

Brief Summary Text - BSTX (15):

The present invention further provides an isolated and purified human **thymosin .beta.15 having the amino acid sequence** of SEC ID NO: 2, or a unique fragment thereof, as well as polypeptides comprising such unique fragments, including, for example, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2.

Brief Summary Text - BSTX (19):

The present invention further provides a method of treating a neoplastic cell expressing human thymosin, .beta.15 by administering to the cell an effective amount of a compound which suppresses the activity or production of the human thymosin .beta.15. Preferably, the compound interferes with the expression of the human **thymosin .beta.15 gene**. Such compounds include, for example, antisense oligonucleotides, ribozymes, antibodies, including single chain antibodies and fragments thereof.

Drawing Description Text - DRTX (2):

FIGS. 1A and 1B show differential mRNA display and Northern analysis of Dunning R-3327 rat prostatic adenocarcinoma variants. Total RNA from AT2.1 (lane 1), AT3.1 (lane 2) and AT6.1 (lane 3) cells were reverse- transcribed and amplified by PCR with a primer set, T.sub.11 AG and a 10 mer AGGGAACGAG (SEQ ID NO:3) in the presence of [.alpha.35-S]dATP. The PCR fragments were displayed on a 6% polyacrylamide gel and autoradiographed. The differentially expressed band is indicated by arrowhead. B. Northern blot analysis of **thymosin .beta.15 gene**. Two .mu.g of poly (A) RNA was isolated from Dunning R-3327 variants AT2. I (lane 1), AT3.1 (lane 2), AT6.1 (lane 3), and Mat Lylu (lane 4), fractionated on a 1.1% formaldehyde-agarose gel, transferred to Hybond-N+ nylon membrane (Amersham) and hybridized with a random primed (Grillon C, et al., FEBS 1990, 274:30-34) .sup.32 P-labeled T.beta.15 T.beta.15 cDNA fragment. The same blot was hybridized with a rat .beta.-actin probe to demonstrate that equivalent amounts of RNA were loaded in each lane.

Drawing Description Text - DRTX (12):

FIGS. 8A and 8B show Western analysis of **thymosin .beta.**-GST fusion protein. FIG. 8A is a Coomasie staining of GST-T.beta. fusion proteins. FIG. 8B is a Western analysis of GST-T.beta. fusion proteins with affinity purified anti-T.beta.15 C-terminal peptide antibody. Lane 1: GST-T.beta.4; Lane 2: GST-T.beta.15; Lane 3: GST only

Detailed Description Text - DETX (2):

A well characterized series of cell lines that show varying metastatic potential has been developed from the Dunning rat prostatic carcinoma (Isaacs, et al., Prostate 9, 261-281 and Bussebakers, et al., Cancer Res. 52,2916-2922 (1992)). Coffey and colleagues previously showed a direct correlation between cell motility and metastatic potential in the Dunning cell lines (Mohler, et al., Cancer Res. 48, 4312-4317 (1988), Parin, et al., Proc. Natl. Acad. Sci, USA 86, 1254-1258 (1989) and Mohler, et al., Cancer Metast. Rev 12, 53-67 (1993)). We compared gene expression in poorly metastatic and highly metastatic cell lines derived from Dunning rat prostate carcinoma using

differential mRNA display. The results of these studies revealed the expression of a novel member of the **thymosin beta** family of actin-binding molecules, thymosin .beta.15. Using this information, we isolated and **sequenced a cDNA enc ding human thymosin** .beta.15.

Detailed Description Text - DETX (3):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that this new member, thymosin .beta.15, directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (4):

The present invention provides a polynucleotide **sequence encoding all or part of thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID NO:2 or a unique fragment thereof. A nucleotide **sequence encoding human thymosin** .beta.15 is set forth as SEQ ID NO:1.

Detailed Description Text - DETX (5):

The sequences of the invention may also be engineered to provide restriction sites, if desired. This can be done so as not to interfere with the peptide **sequence of the encoded thymosin** .beta.15, or may interfere to any extent desired or necessary, provided that the final product has the properties desired.

Detailed Description Text - DETX (27):

The antibody can be administered by a number of methods. One preferred method is set forth by Marasco and Haseltine in PCT WO94/02610, which is incorporated herein by reference. This method discloses the intracellular delivery of a **gene encoding the antibody, in this case the thymosin** .beta.15 antibody. One would preferably use a **gene encoding a single chain thymosin** .beta.15 antibody, The antibody would preferably contain a nuclear localization sequence, for example Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:4) [Lawford, et al. Cell 46:575 (1986)]; Pro-Glu-Lys-Lys-Ile-Lys-Ser (SEQ ID NO:5) [Stanton, et al., Proc. Natl. Acad. Sci. USA 83:1772 (1986)], Gin-Pro-Lys-Lys-Pro (SEQ ID NO:6) [Harlow, et al., Mol. Cell. Biol. 5:1605 (1985)];Arg-Lys-Lys-Arg (SEQ ID NO:7) for the nucleus. One preferably uses an SV40 nuclear localization signal. By this method one can intracellularly express a thymosin .beta.15 antibody, which can block thymosin .beta.15 functioning in desired cells.

Detailed Description Text - DETX (30):

Affecting **thymosin .beta.15 gene** expression may also be achieved more directly, such as by blocking of a site, such as the promoter, on the genomic DNA.

Detailed Description Text - DETX (35):

In addition, ribozymes can be used to inhibit in vitro expression of thymosin .beta.15. For example, the nucleic acids of the invention can further be used to design ribozymes which are capable of cleaving a single-stranded nucleic acid encoding a .beta.15 protein, such as a thymosin .beta.15 mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding **thymosin .beta.15 based upon the sequence** of a nucleic acid of the invention (e.g., SEQ ID NO: 1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base **sequence to be cleaved in a thymosin** .beta.15-encoding mRNA. See for example Cech, et al., U.S. Pat. No. 4,987,071; Cech, et al., U.S. Pat. No. 5,116,742. Alternatively, a nucleic acid of the invention could be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J. W. Science 261,1411-1418 (1993).

Detailed Description Text - DETX (72):

To obtain a full-length complementary DNA (cDNA) clone of this gene, an AT3.1 cDNA library was screened using the originally cloned cDNA fragment from differential display as a probe. A positive clone with a 412 base pair insert was isolated, which contained a single open- reading frame encoding a 45 amino acid protein with a calculated molecular mass of 5304 (FIG. 2). The insert size-of the clone was approximately the same as the molecular size of the transcript seen in Northern analysis suggesting that the clone contained the full length gene sequence. A computer assisted homology search against the Genebank and EMBL DNA databases revealed that the novel gene shared 49% nucleotide sequence homology with rat thymosins .beta.4 and .beta.10. Alignment of the deduced amino acid **sequence of the cloned gene with members of the thymosin .beta**. family (Mihelic, M. & Voelter, Amino Acids 6, 1-13 (1994) showed 68% homology with thymosin .beta.4, 62% with thymosin .beta.10 and 60% with .beta.9, .beta.11 and .beta.12 (FIG. 3). The results suggest that we have cloned a novel .beta. thymosin, now named thymosin .beta.15, from rat prostatic carcinoma cells.

Detailed Description Text - DETX (73):

Hydropathy analysis of the **thymosin .beta.15 protein sequence** revealed no apparent membrane-spanning or membrane-associated regions and no amino-terminal signal sequence. The protein is highly hydrophilic with an estimated isoelectric point of 5.14 and contains regions common to all members of the **thymosin .beta**. family. All .beta.-thymosin family members previously studied, for example, have a putative actin binding region (LKKTET) 16 residues from the amino terminus (Vancompernolle, et al., EMBO J. 11, 4739-4746 (1992), Troys, et al., EMBO J. 15, 201-210(1996). Thymosin 15 also has such a region, although the glutamic acid residue is replaced by an asparagine residue to form

LKKTNT (FIG. 3). The principal region of nonconformity between members of the **thym sin .beta**. family occurs at the carboxyl terminus and the **thymosin .beta.15 sequence** as well shows no significant homology in this region with other family members.

Detailed Description Text - DETX (74):

Members of the .beta.-thymosin family may be independently expressed in different tissues (Lin, et al., J. BioL Chem. 266, 23347-23353 (1991), Voisin, et al. J. Neurochem. 64, 109-120 (1995). Although thymosin .beta.15 is differentially expressed in the prostate carcinoma cell lines tested, all of these lines expressed equivalent levels of thymosins .beta.4 and .beta.10 by RT-PCR analysis (FIG..11). The tissue distribution of thymosin .beta.15 mRNA was examined in the major organs of the rat. No expression of thymosin .beta.15 was detected in the heart, brain, lung, spleen, liver, skeletal muscle and kidney, whereas high expression was found in the testis (FIG. 4). Southern (DNA) analysis of Hind III-, EcoR I- and Pst I-restricted DNA from AT2.1 and AT3.1 cells with thymosin .beta.15 cDNA probe revealed no gross structural alteration of the **thymosin .beta.15 gene** in the tumor cells (data not shown). These results demonstrate that a novel member of the **thymosin .beta**. family is upregulated in metastatic rat prostatic carcinoma cell lines, whereas expression of other **thymosin .beta**. family members (.beta.4 and .beta.10) remains unchanged.

Detailed Description Text - DETX (76):

DNase I digested 5 .mu.g of total RNA from human prostatic carcinoma cell line PC-3 was reverse transcribed using cDNA Cycling Kit (Invitrogen). The reverse transcription mixture was purified with a Spin Column 300 (Pharmocia, Piscataway, N.Y.). 10 .mu.l of purified cDNA reaction was amplified with primers F1 (5'-TATCAGCTAGTGGCTGCACCCGCG-3') (SEQ ID NO:8) and RI (5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR profile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT-PCR were conducted using aliquats from the same samples and amplified with primers to the .beta.-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers was surprisingly 100% identical to the **thymosin .beta.15 sequence** obtained from the rat prostatic carcinoma cells.

Detailed Description Text - DETX (78):

To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified PCR product was 100% identical to the rat **thymosin .beta.15 sequence**. We

conducted in situ hybridization study on samples from patients with varying grades of prostatic carcinomas using a thymosin .beta.15 probe. The tissue sections allowed direct comparison of normal and malignant elements on the same samples. The stromal elements within and around the tumor cell masses, as well as the nonmalignant prostatic epithelium adjacent to the tumor showed little background hybridization with the thymosin .beta.15 antisense probe. In contrast, specific tumor cell islands exhibited a strong specific thymosin .beta.15 signal when probed with antisense (FIG. 5A, small arrow) but not with a sense RNA probe (data not shown). Although nearly all of the tumor cells in the positive islands expressed thymosin .beta.15 mRNA, not all patient specimens were positive and not all islands in a single prostate were positive (FIG. 5A, large arrow). The majority of the negative tumor cells were in non-invasive in situ carcinomas whereas highly invasive tumors were consistently positive (FIG. 5B). Thus a novel .beta. thymosin, first detected in metastatic rat prostate carcinoma cell lines, is upregulated in human prostate cancer.

Detailed Description Text - DETX (82):

To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic expression vector (pcDNA3) containing the **thymosin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thymosin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 7A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 7B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 7C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detailed Description Text - DETX (84):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion proteins with the affinity-purified anti C-terminal antibody. The purified

antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone (FIG. 8) showing its specificity.

Detailed Description Text - DETX (89):

Progression to the metastatic stage is directly correlated with mortality from prostatic carcinoma. It therefore follows that the early diagnosis, prevention, or therapeutic treatment of metastatic progression would lead to more effective control of this disease. The Dunning R-3327 rat prostatic adenocarcinoma model provides several sublines with varying metastatic ability, all of which derive from an original spontaneous tumor and which provide an opportunity to study the steps leading to prostate cancer metastases (Mohler, Cancer Metast. Rev. 12, 53-67 1993) and Pienta, et al. Cancer Surveys 11, 255-263 (1993)). By comparing gene expression among the Dunning cells, we cloned a novel member of the **thymosin .beta**. family, thymosin .beta.15, which is expressed in highly metastatic prostate cancer cells but not in non- or weakly metastatic cells. The related family members thymosin .beta.4 and .beta.10 are expressed equally in all of the cell lines tested such that their expression does not vary with increasing metastatic potential.

US-PAT-NO: 6150117

DOCUMENT-IDENTIFIER: US 6150117 A

TITLE: Method for diagnosis of cancer

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 09/ 135599

DATE FILED: August 18, 1998

PARENT-CASE:

This Application is a divisional of U.S. patent application 08/664,857, filed Jun. 17, 1996, now U.S. Pat. No. 5,858,681.

US-CL-CURRENT: 435/7.1, 435/40.52 , 435/6 , 435/7.23 , 435/91.1 , 435/91.2 , 435/91.21

ABSTRACT:

The present inventors have discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members also directly regulates cell motility in prostatic carcinoma cells. A cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and having the deduced the amino acid sequence (SEQ ID NO: 2) was isolated. The present inventors have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

6 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Abstract Text - ABTX (1):

The present inventors have discovered that humans have a **gene that encodes a n vel protein of the thym sin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members also directly regulates cell motility in prostatic carcinoma cells. A cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and having the deduced the amino acid sequence (SEQ ID NO: 2) was isolated. The present inventors have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (12):

We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Detailed Description Text - DETX (2):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that thymosin .beta.15 directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (10):

Such techniques may include detection with nucleotide probes or may comprise detection of the protein by, for example, antibodies or their equivalent. Preferably, the nucleotide probes may be any that will hybridize more strongly

to the sequence shown in SEQ ID NO: 1 than to other naturally occurring **thymosin sequences**. Types of probe include cDNA, riboprobes, synthetic oligonucleotides and genomic probes. The type of probe used will generally be dictated by the particular situation, such as riboprobes for in situ hybridization, and cDNA for Northern blotting, for example. The most preferred probes are those which correspond to the DNA of SEQ ID NO: 1. Preferably the probe is directed to the thymosin .beta.15 coding region, i.e., nucleotides 98-232 of SEQ ID NO: 1. Most preferably, the probe is directed to nucleotide regions unique to thymosin .beta.15, e.g., nucleotides 113-133, 158-169 or 200-232 of SEQ ID NO: 1. Detection of the **thymosin .beta.15 encoding gene**, per se, will be useful in screening for mutations associated with enhanced expression. Other forms of assays to detect targets more readily associated with levels of expression--transcripts and other expression products will generally be useful as well. The probes may be as short as is required to differentially recognize thymosin .beta.15 mRNA transcripts, and may be as short as, for example, 15 bases.

Detailed Description Text - DETX (49):

(5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR profile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT--PCR were conducted using aliquats from the same samples and amplified with primers to the .beta.-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers is set forth in FIG. 1 (SEQ ID NOS: 1 and 2).

Detailed Description Text - DETX (51):

To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified PCR product was 100% identical to the rat **thymosin .beta.15 sequence**. We conducted in situ hybridization study on samples from patients with varying grades of prostatic carcinomas using a thymosin .beta.15 probe. The tissue sections allowed direct comparison of normal and malignant elements on the same samples. The stromal elements within and around the tumor cell masses, as well as the nonmalignant prostatic epithelium adjacent to the tumor showed little background hybridization with the thymosin .beta.15 antisense probe. In contrast, specific tumor cell islands exhibited a strong specific thymosin .beta.15 signal when probed with antisense (FIG. 3A, small arrow) but not with a sense RNA probe (data not shown). Although nearly all of the tumor cells in the positive islands expressed thymosin .beta.15 mRNA, not all patient specimens were positive and not all islands in a single prostate were positive (FIG. 3A, large arrow). The majority of the negative tumor cells were in non-invasive in situ carcinomas whereas highly invasive tumors were

consistently positive (FIG. 3B). Thus a novel .beta. thymosin, first detected in metastatic rat prostate carcinoma cell lines, is upregulated in human prostate cancer.

Detailed Description Text - DETX (53):

To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic expression vector (pcDNA3) containing the **thymosin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thymosin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 4A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 4B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 4C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detailed Description Text - DETX (55):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone showing its specificity.

US-PAT-NO: 6033860

DOCUMENT-IDENTIFIER: US 6033860 A

TITLE: Expression profiles in adult and fetal organs

DATE-ISSUED: March 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Warrington; Janet A.	Mountain View	CA	N/A	N/A
Nair; Archana	Santa Clara	CA	N/A	N/A

APPL-NO: 09/ 182991

DATE FILED: October 30, 1998

PARENT-CASE:

This application claims benefit of Provisional Application Ser. No. 60/063,857 filed Oct. 31, 1997.

US-CL-CURRENT: 435/6, 536/24.31

ABSTRACT:

Expression profiles have been constructed for liver and brain, in adult and fetal and adolescent tissue. These provide the art with probes which are highly differentially expressed among stages and organs. The profiles and probes can be used to prioritize potential drug targets, to monitor disease progression and remission, and to assess drug metabolism. Solid supports are also provided which facilitate these uses.

22 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Brief Summary Text - BSTX (15):

assaying for the amount of expression in the cell of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone

morphogenetic protein precursor, cytochrome p450-2E1, and **thymosin beta**-10, wherein the expression in the cell is assayed before and after the cell has been contacted with the drug, wherein alteration of the amount of expression of at least one of these genes by the drug is indicative of a deleterious side effect.

Brief Summary Text - BSTX (17):

assaying for expression in the sample of two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and **thymosin beta**-10, wherein expression of G6PD, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, cytochrome p450-2E1, and **thymosin beta**-10 are indicative of an adult liver, and expression of calmodulin, hepatocyte gf, IGF binding protein 1, and ubiquitin are indicative of a fetal liver.

Brief Summary Text - BSTX (19):

assaying for expression in the tissue of two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and **thymosin beta**-10, wherein expression of nicotinic acetylcholine receptor beta 2 or ubiquitin indicates an adult brain.

Brief Summary Text - BSTX (21):

assaying for expression in the tissue of two or more genes selected from the group consisting of: calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and cytochrome p450-2E1, wherein expression of calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, ubiquitin, or bone morphogenetic protein precursor indicates a brain source for the tissue and wherein expression of pyruvate dehydrogenase E1, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, apolipoprotein B100, **thymosin beta**-10, hepatocyte gf, IGF binding protein 1, or cytochrome p450-E1 indicates a liver source for the tissue.

Brief Summary Text - BSTX (23):

assaying for expression in the tissue of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and **thymosin beta**-10, wherein expression of G6PD, calcium channel, synaptotagamin, neuromodulin, **thymosin beta**-10 or bone morphogenetic protein precursor indicates a fetal brain source and expression of pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, indicates a fetal liver source.

Brief Summary Text - BSTX (24):

Another embodiment of the invention provides a solid support for screening a drug for deleterious side effects on a cell. The solid support comprises: at least two oligonucleotides for probing two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and **thymosin beta-10, wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

Brief Summary Text - BSTX (25):

Yet another aspect of the invention is a solid support for distinguishing between a fetal and an adult liver sample. The solid support comprises: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and **thymosin beta-10 wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

Brief Summary Text - BSTX (26):

In mother aspect of the invention a solid support for distinguishing between a fetal and adult brain tissue is provided. The solid support comprises: two more oligonucleotides for detecting two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and **thymosin beta-10, wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

Brief Summary Text - BSTX (29):

Another embodiment of the invention is a solid support for distinguishing a tissue source as fetal brain or fetal liver. The solid support comprises: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and **thymosin beta-10, wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

Detailed Description Text - DETX (11):

Glucose-6-phosphate dehydrogenase deficiency can lead to significant hemolysis. Hemolytic crisis can be induced by exposure to an oxidant, producing profound drops in hematocrit and hemoglobin levels. Calmodulin is a calcium binding protein which controls the assembly of myosin molecules. Calcium channel is involved in ischemic heart disease, stroke and neuronal development and transmission. Neuromodulin is also known as GAP-43. It is involved in neuronal development. Prooncoprotein EWS binds to neuromodulin. VLDLR is the very low density lipoprotein receptor. It is involved in hyper-cholesterolemia. Undulin is involved with kidney diseases, kidney transplantation and hemodialysis. (Clin Nephrol 44(3), 178-184 (1995)). It is

also involved in schistosomiasis and alcoholic liver cirrhosis. Hepatogastroenterology 42(1), 22-26 (1995). Pyruvate dehydrogenase E1 deficiency is associated with Leigh syndrome, microcephaly, and motor neuropathy. Apolipoprotein B100 is involved in atherosclerosis and hypercholesterolemia. Hepatocyte growth factor (gf) is involved in stimulation of the growth of hepatocytes. Insulin-like growth factor-binding protein (IGFBP-3) predisposes breast cancer cells to programmed cell death. Ubiquitin is involved in dendrite outgrowth and differentiation. Cytochrome P450 2E1 is the principal catalyst of human oxidative halothane metabolism in vitro. (J Pharmacol Exp Ther 281(1), 400-411 (1997)). **Thymosin beta**-10 is detected mainly in malignant breast tissue, particularly in the cancerous cells, whereas the normal cell population around the lesions shows very weak staining. Also, the intensity of staining in the cancerous cells was proportionally increased with the increasing grade of the lesions. (Br J Cancer 1996 November; 74(9):1441-1444). In addition, in the highly metastatic human melanoma cell line, BLM, **thymosin beta**-10 correlated with the malignant phenotype. (Biochem Biophys Res Commun 1996 August 23;225(3): 808-816.)

Detailed Description Paragraph Table - DETL (4):
TABLE 4

Accession number	Gene name	Intensity Abrain	Intensity Fbrain	Intensity Aliver	Intensity
Fliver	M12996	G6PD	210	180	70 0
G6PD	T53360	calcium channel	490	350	0 0
CACH	M55047	synaptotagamin	1380	430	0 0
SYNTGN	M25667	neuromodulin	1500	2860	0 0
NEUM	J04046	calmodulin	1500	1510	0 1970
CAM	X53179	nicotinic acetylcholine receptor beta 2	1430	0 0	0
NACB2	D16532	VLDLR	0 0	1300	0
VLDLR	M64108	udulin 1/undulin/excellmatr glycopro	0 0	490	0
XCMG/UN	T65758	pyruvate dehydrogenase E1	230	230	5400
2290	PYDHE1	M10373	apolipoprotein B100	0 0	8520
22850	APOB100	R85613	hepatocyte gf	0 0	400
3790	HPGF	M74587	IGF binding pro1	0 0	570
29520	IGFBP1	T88723	ubiquitin	6900	0
300	42490	UBQ	R71212	bone morphogenetic pro precursor	10800
5560	0 0	BMPP	H46990	cytochrome p450-2E1	0 0
10510	0	CYP2E1	T63133	**thymosin beta**-10	550
3840	2420	0	THYB10		

Claims Text - CLTX (2):
assaying for the amount of expression in the cell of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and **thymosin beta**-10, wherein the expression in the cell is assayed before and after the cell has been contacted with the drug, wherein alteration of the amount of expression of at least one of these genes by the drug is indicative of a deleterious side effect.

Claims Text - CLTX (4):
assaying for expression in the sample of two or more genes selected from the

group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and **thymosin beta**-10, wherein expression of G6PD, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, cytochrome p450-2E1, and **thymosin beta**-10 are indicative of an adult liver, and expression of calmodulin, hepatocyte gf IGF binding protein 1, and ubiquitin are indicative of a fetal liver.

Claims Text - CLTX (6):
assaying for expression in the tissue of two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and **thymosin beta**-10, wherein expression of nicotinic acetylcholine receptor beta 2 or ubiquitin indicates an adult brain.

Claims Text - CLTX (8):
assaying for expression in the tissue of two or more genes selected from the group consisting of: calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and cytochrome p450-2E1, wherein expression of calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, ubiquitin, or bone morphogenetic protein precursor indicates a brain source for the tissue, and wherein expression of pyruvate dehydrogenase E1, VLDLR, udulin1/undulin/extracellular matrix glycoprolein, apolipoprotein B100, **thymosin beta**-10, hepatocyte gf, IGF binding protein 1, or cytochrome p450-E1 indicates a liver source for the tissue.

Claims Text - CLTX (10):
assaying for expression in the tissue of two or more genes selected from the group consisting; of: G6PD, calcium channel, synaptotagamin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and **thymosin beta**-10, wherein expression of G6PD, calcium channel, synaptotagamin, neuromodulin, **thymosin beta**-10 or bone morphogenetic protein precursor indicates a fetal brain source and expression of pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, indicates a fetal liver source.

Claims Text - CLTX (19):
14. A solid support for screening a drug for deleterious side effects on a cell comprising: at least two oligonucleotides for probing two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and **thymosin beta-10, wherein each oligonucleotide comprises a sequence** which is complementary to one

of the two or more genes.

Claims Text - CLTX (20):
15. A solid support for distinguishing between a fetal and an adult liver sample comprising: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and **thymosin beta-10 wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

Claims Text - CLTX (21):
16. A solid support for distinguishing between a fetal and adult brain tissue, comprising: two more oligonucleotides for detecting two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and **thymosin beta-10, wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

Claims Text - CLTX (24):
18. A solid support for distinguishing a tissue source as fetal brain or fetal liver, comprising: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and **thymosin beta-10, wherein each oligonucleotide comprises a sequence** which is complementary to one of the two or more genes.

US-PAT-NO: 6017717

DOCUMENT-IDENTIFIER: US 6017717 A

TITLE: Human **thymosin .beta.15 gene,** protein and uses thereof

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 09/ 069484

DATE FILED: April 29, 1998

PARENT-CASE:

This application is a division of application Ser. No. 08/931,877, filed Sep. 17, 1997, now U.S. Pat. No. 5,831,033, which is a division of application Ser. No. 08/801,796, filed Feb. 14, 1997 now U.S. Pat. No. 5,721,337.

US-CL-CURRENT: 435/7.1, 530/387.1 , 530/387.9 , 530/388.1

ABSTRACT:

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

4 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Abstract Text - ABTX (1):

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein,

herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thym sin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

TITLE - TI (1):
Human **thymosin .beta.15 gene,** protein and uses thereof

Brief Summary Text - BSTX (9):
We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (10):
The present invention provides isolated nucleic acids (polynucleotides) which encode **thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID. NO: 2 or a unique fragment thereof.

Brief Summary Text - BSTX (16):
The present invention further provides an isolated and purified human **thymosin .beta.15 having the amino acid sequence** of SEQ ID NO: 2, or a unique fragment thereof, as well as polypeptides comprising such unique fragments, including, for example, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2.

Brief Summary Text - BSTX (20):
The present invention further provides a method of treating a neoplastic cell expressing human thymosin .beta.15 by administering to the cell an effective amount of a compound which suppresses the activity or production of the human thymosin .beta.15. Preferably, the compound interferes with the expression of the human **thymosin .beta.15 gene**. Such compounds include, for example, antisense oligonucleotides, ribozymes, antibodies, including single chain antibodies and fragments thereof.

Brief Summary Text - BSTX (22):

FIGS. 1A and 1B show differential mRNA display and Northern analysis of Dunning R-3327 rat prostatic adenocarcinoma variants. Total RNA from AT2.1 (lane 1), AT3.1 (lane 2) and AT6.1 (lane 3) cells were reverse-transcribed and amplified by PCR with a primer set, T.sub.11 AG and a 10 mer AGGGAACGAG (SEC ID NO:3) in the presence of [.alpha.35-S]dATP. The PCR fragments were displayed on a 6% polyacrylamide gel and autoradiographed. The differentially expressed band is indicated by arrowhead. B. Northern blot analysis of **thymosin .beta.15 gene**. Two .mu.g of poly (A) RNA was isolated from Dunning R-3327 variants AT2.1 (lane 1), AT3.1 (lane 2), AT6.1 (lane 3), and Mat Lylu (lane 4), fractionated on a 1.1% formaldehyde-agarose gel, transferred to Hybond-N+ nylon membrane (Amersham) and hybridized with a random primed (Grillon C, et al., FEBS 1990, 274:30-34).sup.32 P-labeled T.beta.15 cDNA fragment. The same blot was hybridized with a rat .beta.-actin probe to demonstrate that equivalent amounts of RNA were loaded in each lane.

Brief Summary Text - BSTX (32):

FIGS. 8A and 8B show Western analysis of **thymosin .beta.**-GST fusion protein. FIG. 8A is a Coomasie staining of GST-T.beta. fusion proteins. FIG. 8B is a Western analysis of GST-T.beta. fusion proteins with affinity purified anti-T.beta.15 C-terminal peptide antibody. Lane 1: GST-T.beta.4; Lane 2: GST-T.beta.15; Lane 3: GST only

Detailed Description Text - DETX (2):

A well characterized series of cell lines that show varying metastatic potential has been developed from the Dunning rat prostatic carcinoma (Isaacs, et al., Prostate 9, 261-281 and Bussebakers, et al., Cancer Res. 52,2916-2922 (1992)). Coffey and colleagues previously showed a direct correlation between cell motility and metastatic potential in the Dunning cell lines (Mohler, et al., Cancer Res. 48, 4312-4317 (1988), Parin, et al., Proc. Natl. Acad. Sci, USA 86, 1254-1258 (1989) and Mohler, et al., Cancer Metast. Rev 12, 53-67 (1993)). We compared gene expression in poorly metastatic and highly metastatic cell lines derived from Dunning rat prostate carcinoma using differential mRNA display. The results of these studies revealed the expression of a novel member of the **thymosin beta** family of actin-binding molecules, thymosin .beta.15. Using this information, we isolated and **sequenced a cDNA encoding human thymosin** .beta.15.

Detailed Description Text - DETX (3):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that this new member, thymosin .beta.15, directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (4):
The present invention provides a polynucleotide **sequence encoding all or part of thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID NO:2 or a unique fragment thereof. A nucleotide **sequence enc ding human thymosin** .beta.15 is set forth as SEQ ID NO:1.

Detailed Description Text - DETX (5):
The sequences of the invention may also be engineered to provide restriction sites, if desired. This can be done so as not to interfere with the peptide **sequence of the encoded thymosin** .beta.15, or may interfere to any extent desired or necessary, provided that the final product has the properties desired.

Detailed Description Text - DETX (27):
The antibody can be administered by a number of methods. One preferred method is set forth by Marasco and Haseltine in PCT WO94/02610, which is incorporated herein by reference. This method discloses the intracellular delivery of a **gene encoding the antibody, in this case the thymosin** .beta.15 antibody. One would preferably use a **gene encoding a single chain thymosin** .beta.15 antibody. The antibody would preferably contain a nuclear localization sequence, for example Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:4) [Lawford, et al. Cell 46:575 (1986)]; Pro-Glu-Lys-Lys-Ile-Lys-Ser (SEQ ID NO:5) [Stanton, et al., Proc. Natl. Aced. Sci. USA 83:1772 (1986)], Gln-Pro-Lys-Lys-Pro (SEQ ID NO:6) [Harlow, et al., Mol. Cell. Biol. 5:1605 (1985)]; Arg-Lys-Lys-Arg (SEQ ID NO:7) for the nucleus. One preferably uses an SV40 nuclear localization signal. By this method one can intracellularly express a thymosin .beta.15 antibody, which can block thymosin .beta.15 functioning in desired cells.

Detailed Description Text - DETX (30):
Affecting **thymosin .beta.15 gene** expression may also be achieved more directly, such as by blocking of a site, such as the promoter, on the genomic DNA.

Detailed Description Text - DETX (35):
In addition, ribozymes can be used to inhibit in vitro expression of thymosin .beta.15. For example, the nucleic acids of the invention can further be used to design ribozymes which are capable of cleaving a single-stranded nucleic acid encoding a .beta.15 protein, such as a thymosin .beta.15 mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding **thymosin .beta.15 based upon the sequence** of a nucleic acid of the invention (e.g., SEQ ID NO: 1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base **sequence to be cleaved in a thymosin** .beta.15-encoding mRNA. See for example Cech, et al., U.S. Pat. No. 4,987,071; Cech, et al., U.S. Pat. No. 5,116,742. Alternatively, a nucleic acid of the invention could be used to

select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J. W. Science 261,1411-1418 (1993).

Detailed Description Text - DETX (70):

To obtain a full-length complementary DNA (cDNA) clone of this gene, an AT3.1 cDNA library was screened using the originally cloned cDNA fragment from differential display as a probe. A positive clone with a 412 base pair insert was isolated, which contained a single open- reading frame encoding a 45 amino-acid protein with a calculated molecular mass of 5304 (FIG. 2). The insert size of the clone was approximately the same as the molecular size of the transcript seen in Northern analysis suggesting that the clone contained the full length gene sequence. A computer assisted homology search against the Genebank and EMBL DNA databases revealed that the novel gene shared 49% nucleotide sequence homology with rat thymosins .beta.4 and .beta.10. Alignment of the deduced amino acid **sequence of the cloned gene with members of the thymosin .beta**. family (Mihelic, M. & Voelter, Amino Acids 6, 1-13 (1994) showed 68% homology with thymosin .beta.4, 62% with thymosin .beta.10 and 60% with .beta.9, .beta.11 and .beta.12 (FIG. 3). The results suggest that we have cloned a novel .beta. thymosin, now named thymosin .beta.15, from rat prostatic carcinoma cells.

Detailed Description Text - DETX (71):

Hydropathy analysis of the **thymosin .beta.15 protein sequence** revealed no apparent membrane-spanning or membrane-associated regions and no amino-terminal signal sequence. The protein is highly hydrophilic with an estimated isoelectric point of 5.14 and contains regions common to all members of the **thymosin .beta**. family. All .beta.-thymosin family members previously studied, for example, have a putative actin binding region (LKKTET) 16 residues from the amino terminus (Vancompernolle, et al., EMBO J. 11, 4739-4746 (1992), Troys, et al., EMBO J. 15, 201-210 (1996). Thymosin .beta.15 also has such a region, although the glutamic acid residue is replaced by an asparagine residue to form LKKTNT (FIG. 3). The principal region of nonconformity between members of the **thymosin .beta**. family occurs at the carboxyl terminus and the **thymosin .beta.15 sequence** as well shows no significant homology in this region with other family members.

Detailed Description Text - DETX (72):

Members of the .beta.-thymosin family may be independently expressed in different tissues (Lin, et al., J. Biol. Chem. 266, 23347-23353 (1991), Voisin, et al. J. Neurochem. 64, 109-120 (1995). Although thymosin .beta.15 is differentially expressed in the prostate carcinoma cell lines tested, all of these lines expressed equivalent levels of thymosins .beta.4 and .beta.10 by RT-PCR analysis (FIG. 11). The tissue distribution of thymosin .beta.15 mRNA was examined in the major organs of the rat. No expression of thymosin .beta.15 was detected in the heart, brain, lung, spleen, liver, skeletal muscle and kidney, whereas high expression was found in the testis (FIG. 4). Southern (DNA) analysis of Hind III-, EcoR I- and Pst I-restricted DNA from AT2.1 and AT3.1 cells with thymosin .beta.15 cDNA probe revealed no gross structural alteration of the **thymosin .beta.15 gene** in the tumor cells (data not shown).

These results demonstrate that a novel member of the **thym sin .beta**. family is upregulated in metastatic rat prostatic carcinoma cell lines, whereas expression of other **thymosin .beta**. family members (.beta.4 and .beta.10) remains unchanged.

Detailed Description Text - DETX (74):

DNase I digested 5 .mu.g of total RNA from human prostatic carcinoma cell line PC-3 was reverse transcribed using cDNA Cycling Kit (Invitrogen). The reverse transcription mixture was purified with a Spin Column 300 (Pharmocia, Piscataway, N.Y.). 10 .mu.l of purified cDNA reaction was amplified with primers F1 (5'-TATCAGCTAGTGGCTGCACCCGCG-3') (SEQ ID NO:8) and RI (5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR profile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT-PCR were conducted using aliquats from the same samples and amplified with primers to the .beta.-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers was surprisingly 100% identical to the **thymosin .beta.15 sequence** obtained from the rat prostatic carcinoma cells.

Detailed Description Text - DETX (76):

To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified PCR product was 100% identical to the rat **thymosin .beta.15 sequence**. We conducted in situ hybridization study on samples from patients with varying grades of prostatic carcinomas using a thymosin .beta.15 probe. The tissue sections allowed direct comparison of normal and malignant elements on the same samples. The stromal elements within and around the tumor cell masses, as well as the nonmalignant prostatic epithelium adjacent to the tumor showed little background hybridization with the thymosin .beta.15 antisense probe. In contrast, specific tumor cell islands exhibited a strong specific thymosin .beta.15 signal when probed with antisense (FIG. 5A, small arrow) but not with a sense RNA probe (data not shown). Although nearly all of the tumor cells in the positive islands expressed thymosin .beta.15 mRNA, not all patient specimens were positive and not all islands in a single prostate were positive (FIG. 5A, large arrow). The majority of the negative tumor cells were in non-invasive in situ carcinomas whereas highly invasive tumors were consistently positive (FIG. 5B). Thus a novel .beta. thymosin, first detected in metastatic rat prostate carcinoma cell lines, is upregulated in human prostate cancer.

Detailed Description Text - DETX (80):

To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic expression vector (pcDNA3) containing the **thym sin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thymosin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 7A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 7B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 7C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detailed Description Text - DETX (82):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone (FIG. 8) showing its specificity.

Detailed Description Text - DETX (87):

Progression to the metastatic stage is directly correlated with mortality from prostatic carcinoma. It therefore follows that the early diagnosis, prevention, or therapeutic treatment of metastatic progression would lead to more effective control of this disease. The Dunning R-3327 rat prostatic adenocarcinoma model provides several sublines with varying metastatic ability, all of which derive from an original spontaneous tumor and which provide an opportunity to study the steps leading to prostate cancer metastases (Mohler, Cancer Metast. Rev. 12, 53-67 1993) and Pienta, et al. Cancer Surveys 11, 255-263 (1993)). By comparing gene expression among the Dunning cells, we cloned a novel member of the **thymosin .beta**. family, thymosin .beta.15, which is expressed in highly metastatic prostate cancer cells but not in non- or weakly metastatic cells. The related family members thymosin .beta.4 and .beta.10 are expressed equally in all of the cell lines tested such that their

expression does not vary with increasing metastatic potential.

US-PAT-NO: 5928871

DOCUMENT-IDENTIFIER: US 5928871 A

TITLE: CDNA collections encoding proteins regulated during programmed cell death, and method of use thereof

DATE-ISSUED: July 27, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Heintz; Nathaniel	Pelham Manor	NY	N/A	N/A
Gubbay; John	London	N/A	N/A	GB
Skinner; Michael K.	Pullman	WA	N/A	N/A

APPL-NO: 08/ 925171

DATE FILED: September 8, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The present Application is a Continuation-In-Part of U.S. Ser. No. 08/751,782, filed Nov. 18, 1996, U.S. Pat. No. 5,821,352, the disclosure of which is hereby incorporated by reference in its entirety. Applicants claim the benefits of this Application under 35 U.S.C. .sctn. 120.

US-CL-CURRENT: 435/6, 536/23.5 , 536/24.31

ABSTRACT:

The present invention identifies cDNA collections enriched in genes regulated in prostate homeostasis, prostate regression, and in genes regulated in programmed cell death. These novel cDNA collections provide cDNAs that encode proteins that are either unique to general programmed cell death or unique to prostate regression. These transcripts can be used as markers to screen, monitor and/or to diagnose diseased conditions including prostate cancer. In addition, these cDNA collections can be used in methods of designing novel therapeutic agents useful for treating prostate cancer. Methods of making these collections through subtraction hybridization are described.

41 Claims, 25 Drawing figures

Exemplary Claim Number: 34

Number of Drawing Sheets: 8

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Other Reference Publication - OREF (5):

Wodnar-Filipowicz et al. (1984) Cloning and **sequence analysis f cDNA for rat spleen thymosin beta** 4. Proc. Natl. Acad. Sci. USA 81:2295-2297, Apr. 1984.

US-PAT-NO: 5858681

DOCUMENT-IDENTIFIER: US 5858681 A

TITLE: Method for prognosis of prostate cancer

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 08/ 664857

DATE FILED: June 17, 1996

US-CL-CURRENT: 435/7.1, 435/40.5 , 435/40.52 , 435/6 , 435/7.23 , 435/7.9 , 435/810

ABSTRACT:

The present inventors have discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members also directly regulates cell motility in prostatic carcinoma cells. A cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and having the deduced the amino acid sequence (SEQ ID NO: 2) was isolated. The present inventors have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

5 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Abstract Text - ABTX (1):

The present inventors have discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein

referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members also directly regulates cell motility in prostatic carcinoma cells. A cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and having the deduced the amino acid sequence (SEQ ID NO: 2) was isolated. The present inventors have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (12):

We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Detailed Description Text - DETX (2):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that thymosin .beta.15 directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (10):

Such techniques may include detection with nucleotide probes or may comprise detection of the protein by, for example, antibodies or their equivalent. Preferably, the nucleotide probes may be any that will hybridize more strongly to the sequence shown in SEQ ID NO: 1 than to other naturally occurring **thymosin sequences**. Types of probe include cDNA, riboprobes, synthetic oligonucleotides and genomic probes. The type of probe used will generally be dictated by the particular situation, such as riboprobes for in situ hybridization, and cDNA for Northern blotting, for example. The most preferred

probes are those which correspond to the DNA of SEQ ID NO: 1. Preferably the probe is directed to the thymosin .beta.15 coding region, i.e., nucleotides 98-232 of SEQ ID NO: 1. Most preferably, the probe is directed to nucleotide regions unique to thymosin .beta.15, e.g., nucleotides 113-133, 158-169 or 200-232 of SEQ ID NO: 1. Detection of the **thymosin .beta.15 enc ding gene,** per se, will be useful in screening for mutations associated with enhanced expression. Other forms of assays to detect targets more readily associated with levels of expression--transcripts and other expression products will generally be useful as well. The probes may be as short as is required to differentially recognize thymosin .beta.15 mRNA transcripts, and may be as short as, for example, 15 bases.

Detailed Description Text - DETX (46):

DNase I digested 5 .mu.g of total RNA from human prostatic carcinoma cell line PC-3 was reverse transcribed using cDNA Cycling Kit (Invitrogen). The reverse transcription mixture was purified with a Spin Column 300 (Pharmocia, Piscataway, N.Y.). 10 .mu.l of purified cDNA reaction was amplified with primers F1 (5'-TATCAGCTAGTGGCTGCACCCGCG-3') (SEQ ID NO:8) and RI (5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR profile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT-PCR were conducted using aliquats from the same samples and amplified with primers to the,8-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers is set forth in FIG. 1 (SEQ ID NOS: 1 and 2).

Detailed Description Text - DETX (48):

To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified PCR product was 100% identical to the rat **thymosin .beta.15 sequence**. We conducted in situ hybridization study on samples from patients with varying grades of prostatic carcinomas using a thymosin .beta.15 probe. The tissue sections allowed direct comparison of normal and malignant elements on the same samples. The stromal elements within and around the tumor cell masses, as well as the nonmalignant prostatic epithelium adjacent to the tumor showed little background hybridization with the thymosin .beta.15 antisense probe. In contrast, specific tumor cell islands exhibited a strong specific thymosin .beta.15 signal when probed with antisense (FIG. 3A, small arrow) but not with a sense RNA probe (data not shown). Although nearly all of the tumor cells in the positive islands expressed thymosin .beta.15 mRNA, not all patient specimens were positive and not all islands in a single prostate were positive (FIG. 3A, large arrow). The majority of the negative tumor cells were in non-invasive in situ carcinomas whereas highly invasive tumors were

consistently positive (FIG. 3B). Thus a novel .beta. thymosin, first detected in metastatic rat prostate carcinoma cell lines, is upregulated in human prostate cancer.

Detailed Description Text - DETX (50):

To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic expression vector (pcDNA3) containing the **thymosin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thymosin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 4A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 4B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 4C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detailed Description Text - DETX (52):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone showing its specificity.

Claims Text - CLTX (3):

(B) measuring **thymosin beta** 15 amounts to obtain a **thymosin beta** 15 level in said sample;

Claims Text - CLTX (4):

(C) correlating said **thymosin beta** 15 levels with a baseline level, wherein the baseline level is determined by measuring levels of **thymosin beta** 15 in

disease free individuals;

Claims Text - CLTX (5):

(D) correlating levels of **thym sin beta** 15 at baseline or below, which is a negative result, with a better prognosis than a positive result, wherein the level of **thymosin beta** 15 is above the baseline level.

US-PAT-NO: 5831033

DOCUMENT-IDENTIFIER: US 5831033 A

TITLE: Human **thymosin .beta.15 gene,** protein and uses thereof

DATE-ISSUED: November 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 08/ 931877

DATE FILED: September 17, 1997

PARENT-CASE:

This application is a division of application Ser. No. 08/801,796 filed Feb. 14, 1997, U.S. Pat. No. 5,721,337.

US-CL-CURRENT: 530/387.9, 530/387.1

ABSTRACT:

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

5 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Abstract Text - ABTX (1):
The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is

known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

TITLE - TI (1):
Human **thymosin .beta.15 gene,** protein and uses thereof

Brief Summary Text - BSTX (9):
We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (10):
The present invention provides isolated nucleic acids (polynucleotides) which encode **thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID. NO: 2 or a unique fragment thereof. The term "unique fragment" refers to a portion of the nucleotide sequence or polypeptide of the invention that will contain sequences (either nucleotides or amino acid residues) present in thymosin .beta.15 (SEQ ID NO: 2) but not in other member of the thymosin family. This can be determined when the hybridization profile of that fragment under stringent conditions is such that it does not hybridize to other members of the thymosin family. Such fragments can be ascertained from FIG. 3. A preferred set of unique fragments are those that contain, or contain polynucleotides that encode, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2. Preferably, the unique nucleotide sequence fragment is 10 to 60 nucleotides in length, more preferably, 20 to 50 nucleotides, most preferably, 30 to 50 nucleotides. Preferably, the unique polypeptide sequence fragment is 4 to 20 amino acids in length, more preferably, 6 to 15 amino acids, most preferably, 6 to 10 amino acids.

Brief Summary Text - BSTX (14):
As used herein a polynucleotide "substantially identical" to SEQ ID NO:1 is one comprising at least 90% homology, preferably at least 95% homology, most preferably 99% homology to SEQ ID NO: 1. The reason for this is that such a **sequence can encode thymosin** .beta.15 in multiple mammalian species.

Brief Summary Text - BSTX (15):

The present invention further provides an isolated and purified human **thymosin .beta.15 having the amino acid sequence** of SEQ ID NO: 2, or a unique fragment thereof, as well as polypeptides comprising such unique fragments, including, for example, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2.

Brief Summary Text - BSTX (19):

The present invention further provides a method of treating a neoplastic cell expressing human thymosin .beta.15 by administering to the cell an effective amount of a compound which suppresses the activity or production of the human thymosin .beta.15. Preferably, the compound interferes with the expression of the human **thymosin .beta.15 gene**. Such compounds include, for example, antisense oligonucleotides, ribozymes, antibodies, including single chain antibodies and fragments thereof.

Drawing Description Text - DRTX (2):

FIGS. 1A and 1B show differential mRNA display and Northern analysis of Dunning R-3327 rat prostatic adenocarcinoma variants. Total RNA from AT2.1 (lane 1), AT3.1 (lane 2) and AT6.1 (lane 3) cells were reverse-transcribed and amplified by PCR with a primer set, T.sub.11 AG and a 10 mer AGGGAACGAG (SEQ ID NO:3) in the presence of [.alpha.35-S]dATP. The PCR fragments were displayed on a 6% polyacrylamide gel and autoradiographed. The differentially expressed band is indicated by arrowhead. B. Northern blot analysis of **thymosin .beta.15 gene**. Two .mu.g of poly (A) RNA was isolated from Dunning R-3327 variants AT2.1 (lane 1), AT3.1 (lane 2), AT6.1 (lane 3), and Mat Lylu (lane 4), fractionated on a 1.1% formaldehyde-agarose gel, transferred to Hybond-N+nylon membrane (Amersham) and hybridized with a random primed (Grillon C, et al., FEBS 1990, 274:30-34) .sup.32 P-labeled T.beta.15 cDNA fragment. The same blot was hybridized with a rat .beta.-actin probe to demonstrate that equivalent amounts of RNA were loaded in each lane.

Drawing Description Text - DRTX (12):

FIGS. 8A and 8B show Western analysis of **thymosin .beta.**-GST fusion protein. FIG. 8A is a Coomasie staining of GST-T.beta. fusion proteins. FIG. 8B is a Western analysis of GST-T.beta. fusion proteins with affinity purified anti-T.beta.15 C-terminal peptide antibody. Lane 1: GST-T.beta.4; Lane 2: GST-T.beta.15; Lane 3: GST only

Detailed Description Text - DETX (2):

A well characterized series of cell lines that show varying metastatic potential has been developed from the Dunning rat prostatic carcinoma (Isaacs, et al., Prostate 9, 261-281 and Bussebakers, et al., Cancer Res. 52,2916-2922 (1992)). Coffey and colleagues previously showed a direct correlation between cell motility and metastatic potential in the Dunning cell lines (Mohler, et al., Cancer Res. 48, 4312-4317 (1988), Parin, et al., Proc. Natl. Acad. Sci, USA 86, 1254-1258 (1989) and Mohler, et al., Cancer Metast. Rev 12, 53-67 (1993)). We compared gene expression in poorly metastatic and highly

metastatic cell lines derived from Dunning rat prostate carcinoma using differential mRNA display. The results of these studies revealed the expression of a novel member of the **thymosin beta** family of actin-binding molecules, thymosin .beta.15. Using this information, we isolated and **sequenced a cDNA encoding human thymosin** .beta.15.

Detailed Description Text - DETX (3):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that this new member, thymosin .beta.15, directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (4):

The present invention provides a polynucleotide **sequence encoding all or part of thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID NO:2 or a unique fragment thereof. A nucleotide **sequence encoding human thymosin** .beta.15 is set forth as SEQ ID NO: 1.

Detailed Description Text - DETX (5):

The sequences of the invention may also be engineered to provide restriction sites, if desired. This can be done so as not to interfere with the peptide **sequence of the encoded thymosin** .beta.15, or may interfere to any extent desired or necessary, provided that the final product has the properties desired.

Detailed Description Text - DETX (27):

The antibody can be administered by a number of methods. One preferred method is set forth by Marasco and Haseltine in PCT WO94/02610, which is incorporated herein by reference. This method discloses the intracellular delivery of a **gene encoding the antibody, in this case the thymosin** .beta.15 antibody. One would preferably use a **gene encoding a single chain thymosin** .beta.15 antibody. The antibody would preferably contain a nuclear localization sequence, for example Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:4) [Lawford, et al. Cell 46:575 (1986)]; Pro-Glu-Lys-Lys-Ile-Lys-Ser (SEQ ID NO:5) [Stanton, et al., Proc. Natl. Acad. Sci. USA 83:1772 (1986)], Gln-Pro-Lys-Lys-Pro (SEQ ID NO:6) [Harlow, et al., Mol. Cell. Biol. 5:1605 (1985)]; Arg-Lys-Lys-Arg (SEQ ID NO:7) for the nucleus. One preferably uses an SV40 nuclear localization signal. By this method one can intracellularly express a thymosin .beta.15 antibody, which can block thymosin .beta.15 functioning in desired cells.

Detailed Description Text - DETX (30):
Affecting **thymosin .beta.15 gene** expression may also be achieved more directly, such as by blocking of a site, such as the promoter, on the genomic DNA.

Detailed Description Text - DETX (35):
In addition, ribozymes can be used to inhibit in vitro expression of thymosin .beta.15. For example, the nucleic acids of the invention can further be used to design ribozymes which are capable of cleaving a single-stranded nucleic acid encoding a .beta.15 protein, such as a thymosin .beta.15 mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding **thymosin .beta.15 based upon the sequence** of a nucleic acid of the invention (e.g., SEQ ID NO: 1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base **sequence to be cleaved in a thymosin** .beta.15-encoding mRNA. See for example Cech, et al., U.S. Pat. No. 4,987,071; Cech, et al., U.S. Pat. No. 5,116,742. Alternatively, a nucleic acid of the invention could be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J. W. Science 261,1411-1418 (1993).

Detailed Description Text - DETX (70):
To obtain a full-length complementary DNA (cDNA) clone of this gene, an AT3.1 cDNA library was screened using the originally cloned cDNA fragment from differential display as a probe. A positive clone with a 412 base pair insert was isolated, which contained a single open-reading frame encoding a 45 amino acid protein with a calculated molecular mass of 5304 (FIG. 2). The insert size of the clone was approximately the same as the molecular size of the transcript seen in Northern analysis suggesting that the clone contained the full length gene sequence. A computer assisted homology search against the Genebank and EMBL DNA databases revealed that the novel gene shared 49% nucleotide sequence homology with rat thymosins .beta.4 and .beta.10. Alignment of the deduced amino acid **sequence of the cloned gene with members of the thymosin .beta**. family (Mihelic, M. & Voelter, Amino Acids 6, 1-13 (1994) showed 68% homology with thymosin .beta.4, 62% with thymosin .beta.10 and 60% with .beta.9, .beta.11 and .beta.12 (FIG. 3). The results suggest that we have cloned a novel .beta. thymosin, now named thymosin .beta.15, from rat prostatic carcinoma cells.

Detailed Description Text - DETX (71):
Hydropathy analysis of the **thymosin .beta.15 protein sequence** revealed no apparent membrane-spanning or membrane-associated regions and no amino-terminal signal sequence. The protein is highly hydrophilic with an estimated isoelectric point of 5.14 and contains regions common to all members of the **thymosin .beta**. family. All .beta.-thymosin family members previously studied, for example, have a putative actin binding region (LKKTET) 16 residues from the amino terminus (Vancompernolle, et al., EMBO J. 11, 4739-4746 (1 992), Troys, et al., EMBO J. 15, 201-210 (1996). Thymosin .beta.15 also has such a

region, although the glutamic acid residue is replaced by an asparagine residue to form LKKTNT (FIG. 3). The principal region of nonconformity between members of the **thymosin .beta**. family occurs at the carboxyl terminus and the **thymosin .beta.15 sequence** as well shows no significant homology in this region with other family members.

Detailed Description Text - DETX (72):

Members of the .beta.-thymosin family may be independently expressed in different tissues (Lin, et al., J. Biol. Chem. 266, 23347-23353 (1991), Voisin, et al. J. Neurochem. 64, 109-120 (1995). Although thymosin .beta.15 is differentially expressed in the prostate carcinoma cell lines tested, all of these lines expressed equivalent levels of thymosins .beta.4 and .beta.10 by RT-PCR analysis (FIG. 11). The tissue distribution of thymosin .beta.15 mRNA was examined in the major organs of the rat. No expression of thymosin .beta.15 was detected in the heart, brain, lung, spleen, liver, skeletal muscle and kidney, whereas high expression was found in the testis (FIG. 4). Southern (DNA) analysis of Hind III-, EcoR I- and Pst I-restricted DNA from AT2.1 and AT3.1 cells with thymosin .beta.15 cDNA probe revealed no gross structural alteration of the **thymosin .beta.15 gene** in the tumor cells (data not shown). These results demonstrate that a novel member of the **thymosin .beta**. family is upregulated in metastatic rat prostatic carcinoma cell lines, whereas expression of other **thymosin .beta**. family members (.beta.4 and .beta.10) remains unchanged.

Detailed Description Text - DETX (74):

DNase I digested 5 .mu.g of total RNA from human prostatic carcinoma cell line PC-3 was-reverse transcribed using cDNA Cycling Kit (Invitrogen). The reverse transcription mixture was purified with a Spin Column 300 (Pharmocia, Piscataway, N.Y.). 10 .mu.l of purified cDNA reaction was amplified with primers F1 (5'-TATCAGCTAGTGGCTGCACCCGCG-3') (SEQ ID NO:8) and RI (5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR profile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT-PCR were conducted using aliquats from the same samples and amplified with primers to the .beta.-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers was surprisingly 100% identical to the **thymosin .beta.15 sequence** obtained from the rat prostatic carcinoma cells.

Detailed Description Text - DETX (76):

To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified

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Detailed Description Text - DETX (80):

To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic expression vector (pcDNA3) containing the **thymosin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thymosin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 7A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 7B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 7C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detailed Description Text - DETX (82):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion

proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone (FIG. 8) showing its specificity.

Detailed Description Text - DETX (87):

Progression to the metastatic stage is directly correlated with mortality from prostatic carcinoma. It therefore follows that the early diagnosis, prevention, or therapeutic treatment of metastatic progression would lead to more effective control of this disease. The Dunning R-3327 rat prostatic adenocarcinoma model provides several sublines with varying metastatic ability, all of which derive from an original spontaneous tumor and which provide an opportunity to study. the steps leading to prostate cancer metastases (Mohler, Cancer Metast. Rev. 12, 53-67 1993) and Pienta, et al. Cancer Surveys 11, 255-263 (1993)). By comparing gene expression among the Dunning cells, we cloned a novel member of the **thymosin .beta**. family, thymosin .beta.15, which is expressed in highly metastatic prostate cancer cells but not in non- or weakly metastatic cells. The related family members thymosin .beta.4 and .beta.10 are expressed equally in all of the cell lines tested such that their expression does not vary with increasing metastatic potential.

US-PAT-NO: 5721337

DOCUMENT-IDENTIFIER: US 5721337 A

TITLE: Human thymosin .beta.15

DATE-ISSUED: February 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 08/ 801796

DATE FILED: February 14, 1997

PARENT-CASE:

This is a divisional of copending application Ser. No. 08/664,856 filed on Jun. 17, 1996.

US-CL-CURRENT: 530/300, 530/324 , 530/327

ABSTRACT:

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is direct to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

2 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Abstract Text - ABTX (1):

The present inventors have now discovered that humans have a **gene that enc des a novel protein of th thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is

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Government Interest Text - GOTX (1):
HUMAN **THYMOSIN .beta.15 GENE,** PROTEIN AND USES THEREOF

Brief Summary Text - BSTX (9):
We have now discovered that humans have a **gene that encodes a novel protein f the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members, it also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (10):
The present invention provides isolated nucleic acids (polynucleotides) which encode **thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID. NO: 2 or a unique fragment thereof. The term "unique fragment" refers to a portion of the nucleotide sequence or polypeptide of the invention that will contain sequences (either nucleotides or amino acid residues) present in thymosin .beta.15 (SEQ ID NO: 2) but not in other member of the thymosin family. This can be determined when the hybridization profile of that fragment under stringent conditions is such that it does not hybridize to other members of the thymosin family. Such fragments can be ascertained from FIG. 3. A preferred set of unique fragments are those that contain, or contain polynucleotides that encode, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2. Preferably, the unique nucleotide sequence fragment is 10 to 60 nucleotides in length, more preferably, 20 to 50 nucleotides, most preferably, 30 to 50 nucleotides. Preferably, the unique polypeptide sequence fragment is 4 to 20 amino acids in length, more preferably, 6 to 15 amino acids, most preferably, 6 to 10 amino acids.

Brief Summary Text - BSTX (14):
As used herein a polynucleotide "substantially identical" to SEQ ID NO: 1 is one comprising at least 90% homology, preferably at least 95% homology, most preferably 99% homology to SEQ ID NO: 1. The reason for this is that such a **sequence can encod thymosin** .beta.15 in multiple mammalian species.

Brief Summary Text - BSTX (15):

The present invention further provides an isolated and purified human **thymosin .beta.15 having the amino acid sequence** of SEQ ID NO: 2, or a unique fragment thereof, as well as polypeptides comprising such unique fragments, including, for example, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2.

Brief Summary Text - BSTX (19):

The present invention further provides a method of treating a neoplastic cell expressing human thymosin .beta.15 by administering to the cell an effective amount of a compound which suppresses the activity or production of the human thymosin .beta.15 . Preferably, the compound interferes with the expression of the human **thymosin .beta.15 gene**. Such compounds include, for example, antisense oligonucleotides, ribozymes, antibodies, including single chain antibodies and fragments thereof.

Drawing Description Text - DRTX (2):

FIGS. 1A and 1B show differential mRNA display and Northern analysis of Dunning R-3327 rat prostatic adenocarcinoma variants. Total RNA from AT2.1 (lane 1), AT3.1 (lane 2) and AT6.1 (lane 3) cells were reverse- transcribed and amplified by PCR with a primer set, T.sub.11 AG and a 10 mar AGGGAACGAG (SEQ ID NO:3) in the presence of [.alpha.35-S]dATP. The PCR fragments were displayed on a 6% polyacrylamide gel and autoradiographed. The differentially expressed band is indicated by arrowhead. B. Northern blot analysis of **thymosin .beta.15 gene**. Two//g of poly (A) RNA was isolated from Dunning R-3327 variants AT2. I (lane 1), AT3.1 (lane 2), AT6.1 (lane 3), and Mat Lylu (lane 4), fractionated on a 1.1% formaldehyde-agarose gel, transferred to Hybond-N+nylon membrane (Amersham) and hybridized with a random primed (Grillon C, et al., FEBS 1990, 274:30-34) .sup.32 P-labeled T.beta.15 cDNA fragment. The same blot was hybridized with a rat .beta.-actin probe to demonstrate that equivalent amounts of RNA were loaded in each lane.

Drawing Description Text - DRTX (12):

FIGS. 8A and 8B show Western analysis of **thymosin .beta.**-GST fusion protein. FIG. 8A is a Coomasie staining of GST-T.beta. fusion proteins. FIG. 8B is a Western analysis of GST-T:beta. fusion proteins with affinity purified anti-T.beta.15 C-terminal peptide antibody. Lane 1: GST-T.beta.4; Lane 2: GST-T.beta.15; Lane 3: GST only

Detailed Description Text - DETX (2):

A well characterized series of cell lines that show varying metastatic potential has been developed from the Dunning rat prostatic carcinoma (Isaacs, et al., Prostate 9, 261-281 and Bussebakers, et al., Cancer Res. 52,2916-2922 (1992)). Coffey and colleagues previously showed a direct correlation between cell motility and metastatic potential in the Dunning cell lines (Mohler, et al., Cancer Res. 48, 4312-4317 (1988), Parin, et al., Proc. Natl. Acad. Sci, USA 86, 1254-1258 (1989) and Mohler, et al., Cancer Metast. Rev 12, 53-67 (1993)). We compared gene expression in poorly metastatic and highly

metastatic cell lines derived from Dunning rat prostate carcinoma using differential mRNA display. The results of these studies revealed the expression of a novel member of the **thym sin beta** family of actin-binding molecules, thymosin .beta.15 . Using this information, we isolated and **sequenced a cDNA encoding human thymosin** .beta.15.

Detailed Description Text - DETX (3):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that this new member, thymosin .beta.15, directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (4):

The present invention provides a polynucleotide **sequence encoding all or part of thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID NO:2 or a unique fragment thereof. A nucleotide **sequence encoding human thymosin** .beta.15 is set forth as SEQ ID NO:1.

Detailed Description Text - DETX (5):

The sequences of the invention may also be engineered to provide restriction sites, if desired. This can be done so as not to interfere with the peptide **sequence of the encoded thymosin** .beta.15, or may interfere to any extent desired or necessary, provided that the final product has the properties desired.

Detailed Description Text - DETX (27):

The antibody can be administered by a number of methods. One preferred method is set forth by Marasco and Haseltine in PCT WO94/02610, which is incorporated herein by reference. This method discloses the intracellular delivery of a **gene encoding the antibody, in this case the thymosin** .beta.15 antibody. One would preferably use a **gene encoding a single chain thymosin** .beta.15 antibody. The antibody would preferably contain a nuclear localization sequence, for example Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:4) [Lawford, et al. Cell 46:575 (1986)]; Pro-Glu-Lys-Lys-Ile-Lys-Ser (SEQ ID NO:5) [Stanton, et al., Proc. Natl. Acad. Sci. USA 83:1772 (1986)], Gln-Pro-Lys-Lys-Pro (SEQ ID NO:6) [Harlow, et al., Mol. Cell. Biol 5:1605 (1985)]; Arg-Lys-Lys-Arg (SEQ ID NO:7) for the nucleus. One preferably uses an SV40 nuclear localization signal. By this method one can intracellularly express a thymosin .beta.15 antibody, which can block thymosin .beta.15 functioning in desired cells.

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Affecting **thymosin .beta.15 gene** expression may also be achieved more directly, such as by blocking of a site, such as the promoter, on the genomic DNA.

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In addition, ribozymes can be used to inhibit in vitro expression of thymosin .beta.15. For example, the nucleic acids of the invention can further be used to design ribozymes which-are capable of cleaving a single-stranded nucleic acid encoding a .beta.15 protein, such as a thymosin .beta.15 mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding **thymosin .beta.15 based upon the sequence** of a nucleic acid of the invention (e.g., SEQ ID NO: 1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base **sequence to be cleaved in a thymosin** .beta.15 -encoding mRNA. See for example Cech, et al., U.S. Pat. No, 4,987,071; Cech, et al., U.S. Pat. No. 5,116,742. Alternatively, a nucleic acid of the invention could be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules, See for example Bartel, D. and Szostak, J. W. Science 261,1411-1418 (1993).

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Hydropathy analysis of the **thymosin .beta.15 protein sequence** revealed no apparent membrane-spanning or membrane-associated regions and no amino-terminal signal sequence. The protein is highly hydrophilic with an estimated isoelectric point of 5.14 and contains regions common to all members of the **thymosin .beta**. family. All .beta.-thymosin family members previously studied, for example, have a putative actin binding region (LKKTET) 16 residues from the amino terminus (Vancompernolle, et al., EMBO J. 11, 4739-4746 (1992), Troys, et el., EMBO J. 15, 201-210 (1996). Thymosin .beta.15 also has such a

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Detailed Description Text - DETX (79):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15 . Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion

proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone (FIG. 8) showing its specificity.

Detailed Description Text - DETX (84):

Progression to the metastatic stage is directly correlated with mortality from prostatic carcinoma. It therefore follows that the early diagnosis, prevention, or therapeutic treatment of metastatic progression would lead to more effective control of this disease. The Dunning R-3327 rat prostatic adenocarcinoma model provides several sublines with varying metastatic ability, all of which derive from an original spontaneous tumor and which provide an opportunity to study the steps leading to prostate cancer metastases (Mohler, Cancer Metast. Rev. 12, 53-67 1993) and Pienta, et al. Cancer Surveys 11, 255-263 (1993)). By comparing gene expression among the Dunning cells, we cloned a novel member of the **thymosin .beta**. family, thymosin .beta.15, which is expressed in highly metastatic prostate cancer cells but not in non- or weakly metastatic cells. The related family members thymosin .beta.4 and .beta.10 are expressed equally in all of the cell lines tested such that their expression does not vary with increasing metastatic potential.

Claims Text - CLTX (1):

1. An isolated and purified human **thymosin .beta.15 having the amino acid sequence** set forth in SEQ ID NO.: 2.

US-PAT-NO: 5663071

DOCUMENT-IDENTIFIER: US 5663071 A

TITLE: Human **thymosin .beta. 15 gene,** protein and uses thereof

DATE-ISSUED: September 2, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zetter; Bruce R.	W. Newton	MA	N/A	N/A
Bao; Lere	Brookline	MA	N/A	N/A

APPL-NO: 08/ 664856

DATE FILED: June 17, 1996

US-CL-CURRENT: 435/325, 435/320.1 , 435/358 , 435/365 , 435/367 , 536/23.1 , 536/23.5

ABSTRACT:

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is directed to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2).

10 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Abstract Text - ABTX (1):

The present inventors have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. The present invention is directed to an isolated cDNA encoding the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the

amino acid sequence (SEQ ID NO: 2).

TITLE - TI (1):
Human **thymosin .beta. 15 gene,** protein and uses thereof

Brief Summary Text - BSTX (9):
We have now discovered that humans have a **gene that encodes a novel protein of the thymosin .beta**. family. This novel protein, herein referred to as thymosin .beta.15, has the ability to bind and sequester G-actin, like other members of the **thymosin .beta**. family, but unlike what is known about other members it also directly regulates cell motility in prostatic carcinoma cells. We have isolated a cDNA of the human **thymosin .beta.15 gene** (SEQ ID NO: 1) and have deduced the amino acid sequence (SEQ ID NO: 2). We have shown that enhanced transcripts (mRNA) and expression of the **thymosin .beta.15 gene** in non-testicular cells has a high correlation to disease state in a number of cancers, such as prostate, lung, melanoma and breast cancer, particularly metastatic cancers. Accordingly, discovering enhanced levels of transcript or gene product in non-testicular tissues can be used in not only a diagnostic manner, but a prognostic manner for particular cancers.

Brief Summary Text - BSTX (10):
The present invention provides isolated nucleic acids (polynucleotides) which encode **thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID. NO: 2 or a unique fragment thereof. The term "unique fragment" refers to a portion of the nucleotide sequence or polypeptide of the invention that will contain sequences (either nucleotides or amino acid residues) present in thymosin .beta.15 (SEQ ID NO: 2) but not in other member of the thymosin family. This can be determined when the hybridization profile of that fragment under stringent conditions is such that it does not hybridize to other members of the thymosin family. Such fragments can be ascertained from FIG. 3. A preferred set of unique fragments are those that contain, or contain polynucleotides that encode, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2. Preferably, the unique nucleotide sequence fragment is 10 to 60 nucleotides in length, more preferably, 20 to 50 nucleotides, most preferably, 30 to 50 nucleotides. Preferably, the unique polypeptide sequence fragment is 4 to 20 amino acids in length, more preferably, 6 to 15 amino acids, most preferably, 6 to 10 amino acids.

Brief Summary Text - BSTX (14):
As used herein a polynucleotide "substantially identical" to SEQ ID NO:1 is one comprising at least 90% homology, preferably at least 95% homology, most preferably 99% homology to SEQ ID NO: 1. The reason for this is that such a **sequence can encode thymosin** .beta.15 in multiple mammalian species.

Brief Summary Text - BSTX (15):
The present invention further provides an isolated and purified human

thymosin .beta.15 having the amino acid sequence of SEQ ID NO: 2, or a unique fragment thereof, as well as polypeptides comprising such unique fragments, including, for example, amino acid 7 to 12 of SEQ ID NO: 2, amino acid 21 to 24 of SEQ ID NO: 2 and amino acid 36 to 45 of SEQ ID NO: 2.

Brief Summary Text - BSTX (19):

The present invention further provides a method of treating a neoplastic cell expressing human thymosin .beta.15 by administering to the cell an effective amount of a compound which suppresses the activity or production of the human thymosin .beta.15. Preferably, the compound interferes with the expression of the human **thymosin .beta.15 gene**. Such compounds include, for example, antisense oligonucleotides, ribozymes, antibodies, including single chain antibodies and fragments thereof.

Drawing Description Text - DRTX (2):

FIGS. 1A and 1B show differential mRNA display and Northern analysis of Dunning R-3327 rat prostatic adenocarcinoma variants. Total RNA from AT2.1 (lane 1), AT3.1 (lane 2) and AT6.1 (lane 3) cells were reverse-transcribed and amplified by PCR with a primer set, T.sub.11 AG and a 10 mer AGGGAACGAG (SEQ ID NO:3) in the presence of [.alpha.35-S]dATP. The PCR fragments were displayed on a 6% polyacrylamide gel and autoradiographed. The differentially expressed band is indicated by arrowhead. B. Northern blot analysis of **thymosin .beta.15 gene**. Two .mu.g of poly (A) RNA was isolated from Dunning R-3327 variants AT2. I (lane 1), AT3.1 (lane 2), AT6.1 (lane 3), and Mat Lylu (lane 4), fractionated on a 1.1% formaldehyde-agarose gel, transferred to Hybond-N+ nylon membrane (Amersham) and hybridized with a random primed (Grillon C, et al., FEBS 1990, 274:30-34) .sup.32 P-labeled T.beta.15 cDNA fragment. The same blot was hybridized with a rat .beta.-actin probe to demonstrate that equivalent amounts of RNA were loaded in each lane.

Drawing Description Text - DRTX (12):

FIGS. 8A and 8B show Western analysis of **thymosin .beta.**-GST fusion protein. FIG. 8A is a Coomasie staining of GST-T.beta. fusion proteins. FIG. 8B is a Western analysis of GST-T.beta. fusion proteins with affinity purified anti-T.beta.15 C-terminal peptide antibody. Lane 1: GST-T.beta.4; Lane 2: GST-T.beta.15; Lane 3: GST only

Detailed Description Text - DETX (2):

A well characterized series of cell lines that show varying metastatic potential has been developed from the Dunning rat prostatic carcinoma (Isaacs, et al., Prostate 9, 261-281 and Bussebakers, et al., Cancer Res. 52,2916-2922 (1992)). Coffey and colleagues previously showed a direct correlation between cell motility and metastatic potential in the Dunning cell lines (Mohler, et al., Cancer Res. 48, 4312-4317 (1988), Parin, et al., Proc. Natl. Acad. Sci, U.S.A. 86, 1254-1258 (1989) and Mohler, et al., Cancer Metast. Rev 12, 53-67 (1993)). We compared gene expression in poorly metastatic and highly metastatic cell lines derived from Dunning rat prostate carcinoma using differential mRNA display. The results of these studies revealed the expression of a novel member of the **thymosin beta** family of actin-binding

molecules, thymosin .beta.15. Using this information, we isolated and **sequenced a cDNA encoding human thym sin** .beta.15.

Detailed Description Text - DETX (3):

Although members of the **thymosin .beta**. family have been shown to bind and sequester G-actin, they have not previously been demonstrated to alter cell motility. Our studies, however, reveal that this new member, thymosin .beta.15, directly regulates cell motility in prostatic carcinoma cells. We have shown that expression of thymosin .beta.15 is upregulated in highly metastatic prostate cancer cell lines relative to poorly metastatic or nonmetastatic lines. In addition, thymosin .beta.15 was expressed in human prostate carcinoma specimens but not in normal human prostate. Although not wishing to be bound by theory, this indicates that .beta.15 plays a role in the process of metastatic transformation.

Detailed Description Text - DETX (4):

The present invention provides a polynucleotide **sequence encoding all or part of thymosin .beta.15 having the deduced amino acid sequence** of SEQ ID NO:2 or a unique fragment thereof. A nucleotide **sequence encoding human thymosin** .beta.15 is set forth as SEQ ID NO: 1.

Detailed Description Text - DETX (5):

The sequences of the invention may also be engineered to provide restriction sites, if desired. This can be done so as not to interfere with the peptide **sequence of the encoded thymosin** .beta.15, or may interfere to any extent desired or necessary, provided that the final product has the properties desired.

Detailed Description Text - DETX (27):

The antibody can be administered by a number of methods. One preferred method is set forth by Marasco and Haseltine in PCT WO94/02610, which is incorporated herein by reference. This method discloses the intracellular delivery of a **gene encoding the antibody, in this case the thymosin** .beta.15 antibody. One would preferably use a **gene encoding a single chain thymosin** .beta.15 antibody. The antibody would preferably contain a nuclear localization sequence, for example Pro-Lys-Lys-Lys-Arg-Lys-Val (SEQ ID NO:4) [Lawford, et al. Cell 46:575 (1986)]; Pro-Glu-Lys-Lys-Ile-Lys-Ser (SEQ ID NO:5) [Stanton, et al., Proc. Natl. Acad. Sci. USA 83:1772 (1986)], Gln-Pro-Lys-Lys-Pro (SEQ ID NO:6) [Harlow, et al., Mol. Cell. Biol. 5:1605 (1985)]; Arg-Lys-Lys-Arg (SEQ ID NO:7) for the nucleus. One preferably uses an SV40 nuclear localization signal. By this method one can intracellularly express a thymosin .beta.15 antibody, which can block thymosin .beta.15 functioning in desired cells.

Detailed Description Text - DETX (30):

Affecting **thymosin .beta.15 gene** expression may also be achieved more directly, such as by blocking of a site, such as the promoter, on the genomic

DNA.

Detailed Description Text - DETX (35):

In addition, ribozymes can be used to inhibit in vitro expression of thymosin .beta.15. For example, the nucleic acids of the invention can further be used to design ribozymes which are capable of cleaving a single-stranded nucleic acid encoding a .beta.15 protein, such as a thymosin .beta.15 mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding **thymosin .beta.15 based upon the sequence** of a nucleic acid of the invention (e.g., SEQ ID NO: 1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base **sequence to be cleaved in a thymosin** .beta.15-encoding mRNA. See for example Cech, et al., U.S. Pat. No. 4,987,071; Cech, et al., U.S. Pat. No. 5,116,742. Alternatively, a nucleic acid of the invention could be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J. W. Science 261,1411-1 418 (1993).

Detailed Description Text - DETX (72):

To obtain a full-length complementary DNA (cDNA) clone of this gene, an AT3.1 cDNA library was screened using the originally cloned cDNA fragment from differential display as a probe. A positive clone with a 412 base pair insert was isolated, which contained a single open-reading frame encoding a 45 amino acid protein with a calculated molecular mass of 5304 (FIG. 2). The insert size of the clone was approximately the same as the molecular size of the transcript seen in Northern analysis suggesting that the clone contained the full length gene sequence. A computer assisted homology search against the Genebank and EMBL DNA databases revealed that the novel gene shared 49% nucleotide sequence homology with rat thymosins .beta.4 and .beta.10. Alignment of the deduced amino acid **sequence of the cloned gene with members of the thymosin .beta**. family (Mihelic, M. & Voelter, Amino Acids 6, 1-13 (1994) showed 68% homology with thymosin .beta.4, 62% with thymosin .beta.10 and 60% with .beta.9, .beta.11 and .beta.12 (FIG. 3). The results suggest that we have cloned a novel .beta. thymosin, now named thymosin .beta.15, from rat prostatic carcinoma cells.

Detailed Description Text - DETX (73):

Hydropathy analysis of the **thymosin .beta.15 protein sequence** revealed no apparent membrane-spanning or membrane-associated regions and no amino-terminal signal sequence. The protein is highly hydrophilic with an estimated isoelectric point of 5.14 and contains regions common to all members of the **thymosin .beta**. family. All .beta.-thymosin family members previously studied, for example, have a putative actin binding region (LKKTET) 16 residues from the amino terminus (Vancompernolle, et al., EMBO J. 11, 4739-4746 (1992), Troys, et al., EMBO J. 15, 201-210 (1996). Thymosin .beta.15 also has such a region, although the glutamic acid residue is replaced by an asparagine residue to form LKKTNT (FIG. 3). The principal region of nonconformity between members of the **thymosin .beta**. family occurs at the carboxyl terminus and the **thymosin**

.beta.15 sequence as well shows no significant homology in this region with other family members.

Detailed Description Text - DETX (74):

Members of the .beta.-thymosin family may be independently expressed in different tissues (Lin, et al., J. Biol. Chem. 266, 23347-23353 (1991), Voisin, et al. J. Neurochem. 64, 109-120 (1995). Although thymosin .beta.15 is differentially expressed in the prostate carcinoma cell lines tested, all of these lines expressed equivalent levels of thymosins .beta.4 and .beta.10 by RT-PCR analysis (FIG. 11). The tissue distribution of thymosin .beta.15 mRNA was examined in the major organs of the rat. No expression of thymosin .beta.15 was detected in the heart, brain, lung, spleen, liver, skeletal muscle and kidney, whereas high expression was found in the testis (FIG. 4). Southern (DNA) analysis of Hind III-, EcoR I- and Pst I-restricted DNA from AT2.1 and AT3.1 cells with thymosin .beta.15 cDNA probe revealed no gross structural alteration of the **thymosin .beta.15 gene** in the tumor cells (data not shown). These results demonstrate that a novel member of the **thymosin .beta**. family is upregulated in metastatic rat prostatic carcinoma cell lines, whereas expression of other **thymosin .beta**. family members (.beta.4 and .beta.10) remains unchanged.

Detailed Description Text - DETX (76):

DNase I digested 5 .mu.g of total RNA from human prostatic carcinoma cell line PC-3 was reverse transcribed using cDNA Cycling Kit (Invitrogen). The reverse transcription mixture was purified with a Spin Column 300 (Pharmocia, Piscataway, N.Y.). 10 .mu.l of purified cDNA reaction was amplified with primers F1 (5'-TATCAGCTAGTGGCTGCACCCGCG-3') (SEQ ID NO:8) and RI (5'-AAATGCT GACCTTTCAGTCAGGGT-3') (SEQ ID NO:9) designed to anneal to the outer ends of the **thymosin .beta.15 sequence**. PCR amplification was performed in 50 .mu.l of PCR reaction buffer (50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl.sub.2) with 1 mM of dNTPs, 50 pmol of each primer, and 2.5 U of Taq polymerase (GIBCO BRL), overlaid with 50 .mu.l of mineral oil (Sigma). The PCR profile was 94.degree. C., 30 sec; 60.degree. C., 30 sec; and 72.degree. C., 2 min for 30 cycles. Control studies of the RT-PCR were conducted using aliquats from the same samples and amplified with primers to the .beta.-actin gene (Clontech, Palo Alto, Calif.). Amplification products were separated on 1.6% agarose gels. The amplified PCR product was ligated to pCR using TA cloning kit (Invitrogen, San Diego, (Calif.), and then DNA sequenced. The sequence of the PCR product of human prostatic carcinoma cells amplified by the thymosin .beta.15 primers was surprisingly 100% identical to the **thymosin .beta.15 sequence** obtained from the rat prostatic carcinoma cells.

Detailed Description Text - DETX (78):

To determine whether this thymosin family member may be expressed in human prostate cancer, we examined human prostatic carcinoma cell line PC-3 by RT-PCR with forward and reverse primers for thymosin .beta.15. The PC-3 cells showed a low level of thymosin .beta.15 expression. The DNA sequence of the amplified PCR product was 100% identical to the rat **thym sin .beta.15 sequence**. We conducted in situ hybridization study on samples from patients with varying grades of prostatic carcinomas using a thymosin .beta.15 probe. The tissue

sections allowed direct comparison of normal and malignant elements on the same samples. The stromal elements within and around the tumor cell masses, as well as the nonmalignant prostatic epithelium adjacent to the tumor showed little background hybridization with the thymosin .beta.15 antisense probe. In contrast, specific tumor cell islands exhibited a strong specific thymosin .beta.15 signal when probed with antisense (FIG. 5A, small arrow) but not with a sense RNA probe (data not shown). Although nearly all of the tumor cells in the positive islands expressed thymosin .beta.15 mRNA, not all patient specimens were positive and not all islands in a single prostate were positive (FIG. 5A, large arrow). The majority of the negative tumor cells were in non-invasive in situ carcinomas whereas highly invasive tumors were consistently positive (FIG. 5B). Thus a novel .beta. thymosin, first detected in metastatic rat prostate carcinoma cell lines, is upregulated in human prostate cancer.

Detailed Description Text - DETX (82):

To determine whether thymosin .beta.15 expression had an effect on cell motility, we transfected highly motile AT3.1 cells with a eukaryotic expression vector (pcDNA3) containing the **thymosin .beta.15 gene** in antisense orientation driven by the constitutive human cytomegalovirus promoter. The transfected cells growing in selective (G418) media were examined for expression of antisense transcripts of the **thymosin .beta.15 gene** by strand-specific polymerase chain reaction (PCR) amplification (Zhou, et al., Cancer Res. 52, 4280-4285 (1992). Analysis of cell motility in a multiwell Boyden chamber apparatus (Boyden, S. V., J. Exp. Med. 115, 453-466 (1962)) using fetal bovine serum as a migration stimulus revealed that the motility of the transfectants which showed expression of antisense transcripts was significantly reduced relative to the vector-only controls (FIG. 7A). Two antisense transfected clones which did not express antisense transcripts failed to show any decreased rate of cell motility (data not shown). In a further experiment, poorly motile AT2.1 cells, transfected with sense thymosin .beta.15 constructs and confirmed to express thymosin .beta.15 by Northern analysis, were shown to have significantly increased stimulated motility relative to their vector controls (FIG. 7B). Both the sense and antisense thymosin .beta.15 transfectants showed similar rates of cell proliferation relative to controls suggesting differential specificity for different cellular events (FIG. 7C). The results demonstrate that thymosin .beta.15, which is upregulated in the highly motile AT3.1 and AT6.1 Dunning tumor cell lines, is a positive regulator of cell motility which is an important component of cancer metastasis.

Detailed Description Text - DETX (84):

A polyclonal antibody was raised against a peptide representing the 11 C-terminal amino acids of thymosin .beta.15. Synthesized peptide was coupled with a carrier, keyhole limpet hemocyanin (KLH), and injected into rabbits. Antiserum was affinity-purified over the C-terminal peptide coupled CNBr-activated sepharose 4B column. To test the specificity of the purified antibody, we performed Western analysis of the GST/**thymosin .beta**. fusion proteins with the affinity-purified anti C-terminal antibody. The purified antibody strongly reacted with GST-thymosin .beta.15 fusion protein, but did not cross react with GST-thymosin .beta.4, and not with GST alone (FIG. 8)

showing its specificity.

Detailed Description Text - DETX (89):

Progression to the metastatic stage is directly correlated with mortality from prostatic carcinoma. It therefore follows that the early diagnosis, prevention, or therapeutic treatment of metastatic progression would lead to more effective control of this disease. The Dunning R-3327 rat prostatic adenocarcinoma model provides several sublines with varying metastatic ability, all of which derive from an original spontaneous tumor and which provide an opportunity to study the steps leading to prostate cancer metastases (Mohler, Cancer Metast. Rev. 12, 53-67 1993) and Pienta, et al. Cancer Surveys 11, 255-263 (1993)). By comparing gene expression among the Dunning cells, we cloned a novel member of the **thymosin .beta**. family, thymosin .beta.15, which is expressed in highly metastatic prostate cancer cells but not in non- or weakly metastatic cells. The related family members thymosin .beta.4 and .beta.10 are expressed equally in all of the cell lines tested such that their expression does not vary with increasing metastatic potential.

Claims Text - CLTX (1):

1. An isolated polynucleotide encoding human **thymosin .beta.15 comprising the amino acid sequence** as set forth in SEQ ID NO:2.

Claims Text - CLTX (7):

7. An isolated polynucleotide encoding human **thymosin .beta.15 having the nucleotide sequence** of nucleotides 98-232 of SEQ ID NO:1, or the complement thereto.

Other Reference Publication - OREF (2):

Rudin et al., Differential splicing of **thymosin .beta.**-4 mRNA, J. Immunol., vol. 144(12), pp. 4857-4862 Jun. 1990.

US-PAT-NO: 4297276

DOCUMENT-IDENTIFIER: US 4297276 A
See image for Certificate of Correction

TITLE: **Thymosin beta** 3 and beta 4

DATE-ISSUED: October 27, 1981

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goldstein; Allan L.	Washington	DC	N/A	N/A
Low; Teresa L. K.	Annandale	VA	N/A	N/A

APPL-NO: 06/ 159430

DATE FILED: June 16, 1980

PARENT-CASE:

RELATED APPLICATIONS

This application is a continuation-in-part of copending application Ser. No. 23,115, filed Mar. 23, 1979, now abandoned which is a continuation-in-part of Ser. No. 967,675, filed Dec. 8, 1978, now abandoned.

US-CL-CURRENT: 530/324, 930/180 , 930/DIG.750

ABSTRACT:

Two related polypeptides, thymosin .beta..sub.3 and thymosin .beta..sub.4, have been isolated from Thymosin fraction 5. These peptides have been characterized and sequenced. Thymosin .beta..sub.3 has 50 amino acid residues while thymosin .beta..sub.4 has 43 amino acid residues corresponding identically to the amino terminal 43 amino acids of thymosin .beta..sub.3. The compounds have useful biological activity as evidenced by their ability to induce terminal deoxynucleotidyl transferase (TdT) positive cells in T-cell populations. The invention described herein was made in part in the course of work under a grant or award from the Department of Health, Education and Welfare.

2 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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TITLE - TI (1):
Thym sin beta 3 and beta 4

Brief Summary Text - BSTX (2):
The isolation and characterization of a peptide component of thymosin fraction 5 termed thymosin .alpha..sub.1, was described in U.S. Pat. No. 4,079,127. Thymosin .alpha..sub.1 contained 28 amino acid residues and was an acidic peptide having a pI of about 4.0-4.3. It is further distinguished in having a blocked amino terminal (N-acetyl). Biologically thymosin .alpha..sub.1 is active in the MIF, E-rosette and mitogen assays but is not active in the mixed lymphocyte response (MLR) assay. The amino acid **sequence for thymosin** .alpha..sub.1 is as follows:

Brief Summary Text - BSTX (5):
The **sequence for thymosin** .beta..sub.3 and .beta..sub.4 advanced in the parent application, Ser. No. 967,675, has been revised in amino acids 24-35 by exchanging original sequence 30-35 for original sequence 24-29 of both peptides. This revision is based on additional data derived from thermolysin digests used in the peptide mapping.

Detailed Description Text - DETX (2):
The present invention relates to the isolation and chemical characterization of thymosin .beta..sub.3 and .beta..sub.4, Thymosin .beta..sub.3 has an isoelectric point of 5.2 and a molecular weight of 5,500. Thymosin .beta..sub.4 has an isoelectric point of 5.1 and molecular weight of 4,982. They are the only two polypeptides isolated from fraction 5 thus far that can induce TdT positive cells. The induction of TdT by thymosin fraction 5 exhibits a bell shaped dose response curve. However, TdT response to thymosin .beta..sub.3 and .beta..sub.4 increases as the doses increase. The amino acid **sequences for thymosin** .beta..sub.3 and .beta..sub.4 are as follows:

Detailed Description Text - DETX (3):
Sequence of Thymosin .beta..sub.3 ##STR1## -Lys-Phe-Asp-Lys-Ser.sup.15 -Lys--Leu-Lys-Lys-Thr.sup.20 -Glu-Thr-Gln-Glu-Lys.sup.25 -Asn-

Detailed Description Text - DETX (6):
Sequence of Thymosin .beta..sub.4 ##STR2## -Lys-Phe-Asp-Lys-Ser.sup.15 -Lys--Leu-Lys-Lys-Thr.sup.20 -Glu-Thr-Gln-Glu-Lys.sup.25 -Asn

Detailed Description Text - DETX (22):
These peptides have been sequenced and the location of acids or amides assigned. These data, along with results obtained from cyanogen bromide cleavage of thymosin .beta..sub.4 as well as partial acid hydrolysis of CNBr fragment 1, established the **sequence of N-terminal 14-residue of thymosin** .beta..sub.4 as follows:

Detailed Description Text - DETX (42):
Sequencer Run of Thymosin .beta..sub.3

Detailed Description Text - DETX (43):
A sample of the cyanogen bromide (CNBr) clearage product of **thymosin .beta..sub.3 was applied to a sequencer** (Beckman 890 c). The sample was precoupled with sulfophenylisothiocyanate (3-SPITC) in the reaction cup before the sequencer program was initiated. Beckman DMAA program (peptide program 102974) was used. The sequencer products were identified by high performance liquid chromatograph (HPLA) in a Hewlett Packard 1084B and/or analyzed by amino acid analysis after backhydrolysis with hydriodic acid. The results which are in total agreement with data obtained by manual sequence techniques are as follows:

Detailed Description Text - DETX (46):
Amino Acid **Sequence of Thymosin Beta** 4 ##STR3## -Lys-Phe-Asp-Lys-Ser.sup.15 -Lys-Leu-Lys-Lys-Thr.sup.20 -Glu-Thr-Gln-Glu-Lys.sup.25 -Asn-Pro-Leu-Pro-Ser.sup.30 -Lys-Glu-Thr-Ile-Glu.sup.35 -Gln-Glu-Lys-Gln-Ala.sup.40 -Gly-Glu-Ser-OH.

Detailed Description Text - DETX (47):
Amino Acid **Sequence of Thymosin Beta** 3 ##STR4## -Lys-Phe-Asp-Lys-Ser.sup.15 -Lys-Leu-Lys-Lys-Thr.sup.20 -Glu-Thr-Gln-Glu-Lys.sup.25 -Asn-Pro-Leu-Pro-Ser.sup.30 -Lys-Glu-Thr-Ile-Glu.sup.35 -Gln-Glu-Lys-Gln-Ala.sup.40 -Gly-Glu-Ser-(Asx, Glx.sup.45, Ile, Thr)Ala-Lys-Thr.sup.50 -OH.